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THE BRITISH JOURNAL

OF

EXPERIMENTAL PATHOLOGY

IS HÆMOLYSED BLOOD TOXIC?

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STATEMENTS have been made from time to time that when hæmoglobin is set free into the plasma it acts as a toxic substance. Further, that the mere defibrination of blood by gentle whipping, which causes the destruction of very few corpuscles, renders its re-injection fatal or very dangerous. Again, the presence in the blood of various substances, such as bile-salts, nitrites, certain aromatic compounds, as aniline or pyrogallol, converts the hæmoglobin into methæmoglobin to a greater or less degree. This change may or may not be accompanied by destruction of the corpuscles with passage of their hæmoglobin or methæmoglobin into solution in the plasma. The serious results of transfusion of blood into a patient whose blood hæmolyses the corpuscles of that introduced are apparently regarded as due to the hæmolysis as such.

Certain experiments which I had occasion to make recently and in which destruction of blood-corpuscles was involved showed an unexpected absence of toxic effects. Further doubt with regard to the truth of the received interpretations was aroused by a letter which I received from Dr. Burkitt, of Nairobi, who had succeeded in the recovery of "hopeless" cases of black-water fever by the intravenous injection of gum-saline, which was found to raise the blood-pressure and restore the secretion of urine. It is difficult to

believe that so inert a substance as gum acacia could have any remedial action other than that due indirectly to the increase of the volume and pressure of the blood.

The question seemed worth some further investigation. We may first consider the results of mere defibrination of the blood. It is well known that the re-injection of defibrinated blood of a rabbit, even into the same animal from which it was taken, is followed by death. This was found to be explained by the production of intravascular clotting. Whether this is due to the exceptional properties of rabbit's blood or to a peculiar propensity of this animal to intravascular clotting is difficult to decide, on account of the toxic properties of blood from a different species in the case of animals in general. Brodie (1) ascribed these toxic properties to a protein in the serum produced in the act of coagulation. It is to be noted, however, that the remarkable properties of foreign proteins, of which anaphylaxis is one manifestation, were unknown at the time of his experiments, only one of which consisted of the injection into a cat of its own defibrinated blood. All the others were with foreign serum. I am inclined to attribute the toxic action obtained in the one case of the defibrinated blood of the cat itself to some accidental circumstance, since I have not met with it in a large number of experiments in which the defibrinated blood of the same or another cat was injected for various purposes. We may conclude that, with the exception of the rabbit, which is peculiarly sensitive to the "fibrin-ferment" produced in clotting, the re-injection of defibrinated blood into the animal itself is harmless. Whether man does or does not behave like the rabbit I am unable to say, not having met with any case in which defibrinated blood has been injected. It appears, however, from some remarks by Quénu (2), that blood-serum has been used in the French Army for the treatment of wound-shock. But no data are given as to the results.

We may pass on to the more interesting problem of hæmolysis.

Couvreur and Clément (3) describe experiments in which they re-injected rabbit's blood, after the corpuscles had been laked, into the same animal from which the blood had been taken. It is unfortunate, for the reason given above, that rabbits were used, and the description of the manner of death—convulsions, with a loud cry—suggests that intravascular clotting had been produced. It is true that one experiment is given in which the serum of clotted blood was innocuous, but it is to be remembered that individual rabbits may be met with in which the phenomenon of intravascular clotting is absent. Mudge (4), indeed, states that albino rabbits are refractory in this respect; but it is doubtful whether his experiments were sufficiently numerous to warrant a general statement.

An experiment made by Paul Bert (5) is given by himself as a proof that hæmoglobin freed from the corpuscles is toxic. A dog was bled until rigidity of the limbs came on. The injection of the blood removed, after it had been

frozen and thawed, failed to restore life—in fact, it is stated that death ensued more rapidly than if serum alone had been used. It will be clear that the data are scarcely sufficient to justify the conclusion made. We are not told how long the animal had remained with the very low blood-pressure due to the hæmorrhage, and it is possible that the nerve centres had become irrecoverable. The procedure was too drastic to test the question.

Roger (6) states that, while freshly laked blood is toxic to rabbits, and in doses of 4 to 6·8 c.c. per kilo., it loses this property if allowed to undergo autolysis in an incubator for ten days. These results do not permit any conclusion to be drawn as to whether it was the fibrin-ferment that had disappeared, or whether any other toxic constituent was present in the fresh blood. So far as they go they do not support the view that hæmoglobin itself is toxic. It would probably still be present, although deprived of oxygen, in the autolysed blood, assuming that it remained aseptic.

Barratt (7) also made experiments on the rabbit. He came to the conclusion that the effects of hæmolysed blood were due to intravascular clotting, brought about by the presence in the serum of "thrombokinase," liberated from the stromata of the red cells. He states that he could obtain no evidence of any other toxic constituent.

The suppression of urine in black-water fever has been attributed to the hæmolysis present, but Yorke and Nauss (8) showed that, even in rabbits, hæmolysis alone had not this result, and that it was fall of blood-pressure that was responsible for it. If this happened, the lumen of the renal tubules became clogged with granules of hæmoglobin, doubtless the result of absorption of water from the solution of hæmoglobin filtering through the glomeruli. As the blood-pressure was low this filtrate would be scanty, and in its slow passage along the tubules would lose the greater part of its water by reabsorption. It seems probable that the restoration of renal secretion in Burkitt's cases was due indirectly to the rise in blood-pressure.

My experiments on the question were made with the object of testing whether destruction of red blood-corpuscles was accompanied by the passage of toxic substances into the blood similar to those produced in injured tissues, and having an action similar to that of histamine, as described by Dale and his coadjutors (9, 10). I was unaware at the time of the results of Barratt and of Yorke and Nauss, and therefore somewhat surprised to find that blood hæmolysed by freezing and thawing was not only innocuous to cats, but raised the blood-pressure, if it was low, and increased the secretion of urine. A volume of blood amounting to about one-third of that calculated as being present was first withdrawn, and replaced by an equal volume of gum-saline in order to keep up a normal blood-pressure. The blood was defibrinated by whipping, frozen at $-15°$, and thawed. It appeared to be completely hæmolysed. Sometimes there is a second feeble coagulation on thawing, which makes it advisable to whip the blood gently with a feather during the

thawing. Straining through muslin is sufficient to remove small clots, etc. The stromata, not being disintegrated by this process, are reinjected in the hæmolysed blood. The intravenous injection of 18 c.c. of blood so prepared into a cat of 1·9 kilos. weight had no other result than a slight permanent rise in the blood-pressure. The urine, which was, of course, stained with hæmoglobin, was somewhat increased in amount, probably owing to the rise in blood-pressure. It amounted to 1 c.c. in five minutes—a good value for an anæsthetised cat. In order to find out what degree of hæmolysis existed, the total hæmoglobin content in a sample of the blood was compared, by Sahli's method, with that free in the plasma. The latter was determined by diluting 1 c.c. of the blood with 99 c.c. of Ringer's solution, allowing the corpuscles to deposit and estimating the hæmoglobin in the supernatant liquid. It was found that 22 per cent. of the total hæmoglobin was free in the plasma. If all the corpuscles injected had been hæmolysed the calculated amount would be 20 per cent., so that it is evident that once freezing and thawing is effective for complete hæmolysis.

In another experiment the stromata were separated from the hæmolysed blood by the centrifuge, the deposit extracted with dilute alkali, neutralised and filtered. There was no fall of blood-pressure nor other toxic action when injected. Finally, the deposit on the filter-paper was suspended in Ringer's solution and injected, with no result.

It should be mentioned that in one case only out of many the injection of hæmolysed blood was found to produce intravascular clotting. This was clearly due to a peculiar individual sensibility to fibrin ferment.

Some interesting facts came out in another set of experiments in which hæmolysis was produced *in vivo* by the intravenous injection of warmed tap-water. A volume of water equal to the calculated volume of the blood-plasma was introduced slowly. In one case, in which seventy-two minutes were taken for the injection, a sample of blood taken five minutes after the end of the injection showed a total hæmoglobin content of 98·8 per cent. of the original value, so that practically all of the water injected had left the circulation. There was no fall in the blood-pressure. Indeed, it seemed to me that water was more effective in maintaining the blood-pressure than a saline solution. Notwithstanding the fact that there was no fall in the blood-pressure, the rate of the urinary flow was only one-quarter of its initial rate. It contained much hæmoglobin, in concentration one-third of that in the plasma. The percentage of hæmolysed corpuscles was found to be between one-fifth and one-sixth of the whole. In another similar experiment there was no actual diminution in the renal secretion—rather a slight increase, but very much less than would have resulted from an equal volume of Ringer's solution. That this action on the kidney was in some way connected with the dilution of the salts of the plasma, and not with the presence of hæmoglobin in solution, is shown by the result of adding sodium chloride to the blood in

circulation. Although the water injected had left the blood-vessels, it is to be remembered that the total salt concentration of the blood and tissues was distributed over a much larger volume of liquid than normally, so that the plasma would still be hypotonic. The amount of sodium chloride required to make the water introduced into a 0·9 per cent. solution was dissolved in a very small quantity of water and injected into a vein. Although this was done very slowly the result was a rather large fall of blood-pressure—a common result in my experience of hypertonic saline solutions. This fall was recovered from in the course of twenty minutes, and the urinary flow was then raised to three times its original rate. It may be noted that Regéczy (11) injected large quantities of water into the veins of dogs without apparent harm. The explanation of the decreased urinary secretion when the salt-content of the plasma is below normal may possibly be due to the increase of osmotic pressure of the plasma proteins, thus reducing the effective filtration pressure in the glomeruli. I found (12) that the osmotic pressure of the proteins of ox-serum was 36–40 mm. of mercury in the presence of Ringer's solution, 116 mm. in water.

It is well known that certain chemical substances, such as nitrites, pyrogallol and other benzene derivatives, bile-salts and so on, change a part of the hæmoglobin into methæmoglobin, shown by the brown colour of the blood. I tested the action of some of these, namely pyrogallol, aniline acetate and bile-salts. They showed themselves to be toxic by the fall of blood-pressure and certain nervous effects. In the case of pyrogallol, which was given in the somewhat excessive dose of 0·8 grm. per kilo., the respiratory centre was paralysed. The blood became brown in colour but there was no actual breaking up of the corpuscles. The urine was greatly reduced in amount but normal in colour. The reduction in the kidney secretion was evidently due to the lowered blood-pressure, because it was restored more or less by the injection of gum-saline, which had the effect of a partial restoration of the blood-pressure. There appears to have been sufficient unaltered hæmoglobin to convey oxygen to the tissues if it was carried round fast enough by increasing the volume of the blood. Since there was no actual hæmolysis in these experiments, they only bear indirectly on the question at issue. Saponin, which does actually cause the passage of hæmoglobin into the plasma, was found to stop the heart before any notable degree of hæmolysis had been produced.

Although methæmoglobin can be reconverted into hæmoglobin by a chemical reducing agent, it appears that merely reducing the oxygen tension is incapable of doing this. When once formed it is a useless substance in the body and has to be got rid of.

Hæmolysis in itself appears, then, to be harmless. The ill-effects ascribed to it must be due to other concurrent phenomena. For example, the results of transfusion of incompatible blood are probably due to a reaction to foreign

protein, analogous to that of anaphylactic shock. In this connection the statements of Schmidt and Bennett (13) are of interest. They show that hæmoglobin itself is unable to act as an antigen, whereas the "globin" obtained from it by chemical agency has this property. Brodie (14) found that foreign blood is less effective in the perfusion of isolated organs, especially the heart, than that of the same animal is. There is produced in some way an obstruction to the flow. Cushny and Gunn (15) showed that serum and egg-white have a peculiar constricting effect on the coronary arteries of a heart that has been previously perfused with a pure saline solution. Yanagawa (16) showed that the property is associated with the albumen of the serum, not with the globulin. Of course hæmoglobin in solution in the plasma is lost by the urine, and if there is a fall in blood-pressure from other causes, as by loss of fluid from the blood, there may be obstruction of renal tubules. The practical conclusion to be drawn is, obviously, that the blood-pressure must be kept up by intravenous injection, not by vaso-constrictor drugs, which really reduce the supply to the capillaries.

Although hæmoglobin shows itself to be innocuous when free in the plasma, it does not necessarily follow that it can serve its normal purpose of carrying oxygen. It is true that Barcroft and his coadjutors have been unable to find any difference in the relations of hæmoglobin to oxygen, whether they investigated the corpuscles or a solution.

It is well known that a solution devoid of hæmoglobin is insufficient to supply enough oxygen to many organs, especially glands, when used for artificial perfusion. Dale and Richards (9) found also that the normal dilator reaction of the capillaries to histamine was absent unless red blood-corpuscles were present in the perfusion fluid. I have used this latter fact as a test for the oxygen-carrying capacity of dissolved hæmoglobin. Cats were used for the experiments. Blood was first removed from a cannula in one of the carotid arteries to the extent of about half the calculated volume in the body. It was immediately replaced by gum-saline in order to keep the animal alive. The blood withdrawn was defibrinated by whipping and hæmolysed by the addition of twice its volume of a 6·5 per cent. solution of gum acacia in distilled water. Sodium chloride was then added in sufficient amount to make the gum solution present up to 0·9 per cent. The mixture was strained through glass wool and adrenaline added to make one part in five millions, as shown by Dale and Richards to be necessary to give tone to the capillaries. The femoral artery and vein, having been already prepared, were tied, a cannula, previously connected with the perfusion apparatus, and containing the oxygenated hæmoglobin solution, rapidly inserted, and the vein opened to allow the fluid to escape. The cat was then killed by opening the heart, and the leg inserted into a plethysmograph containing warm water, and arranged to trace on a smoked surface by means of a float recorder in the usual manner. The injection of 0·1 mgrm. of histamine into the rubber tube near

the cannula was followed in some cases by an obvious dilatation of the limb, showing that dissolved hæmoglobin acts as an efficient oxygen carrier. Whether it is equally efficient in this respect to the red corpuscles is not, of course, shown. It is to be remembered that, as Dale and Richards pointed out, the action of histamine shows itself in the plethysmograph as a difference between constriction of arterioles and dilatation of capillaries, and may therefore vary much in different cases. It seems, therefore, that unless there is actual decrease in volume we must suppose that the capillaries reacted. If the perfusion fluid contains no hæmoglobin the arterial constriction alone is seen. This was the case in one of my experiments, in which the circulation in the limb had been interrupted too long, owing to the death of the animal from an overdose of ether.

It was found that the excitability of the muscles on stimulation of the sciatic nerve was prolonged for a greater time when perfused with a solution containing hæmolysed blood, as compared with that of the opposite side. Although this observation may perhaps indicate no more than that perfused muscles retain their excitability longer than those through which no circulation takes place, it shows that hæmolysed blood was not toxic. The isolated mammalian heart, perfused with a solution containing salts and gum arabic, showed an increase in magnitude of beat when hæmolysed blood was added. But this was only temporary and in all these perfusion experiments the vaso-constrictor action of the serum-albumin, to which reference has already been made, sooner or later causes a great decrease in the rate of flow through organs when solutions containing whole blood, serum or plasma are used. In future experiments, the effect of blood-corpuscles which have been washed with saline before hæmolysis must be tested.

The advantage of the possibility of using hæmoglobin in solution in perfusion fluids is that, when corpuscles are added, there is a tendency for these to deposit in parts of the apparatus where its rate of flow is small or at a lower level. This is especially the case when gum acacia is present, as noted by Dale and Richards. The fact is of importance in two ways: the circulating fluid becomes more or less deprived of the advantage of the hæmoglobin, and, owing to the changes in viscosity, brought about by the irregular distribution of the corpuscles, observations on the rate of flow through perfused organs are made inaccurate.

SUMMARY.

Experiments with defibrinated or hæmolysed blood on the rabbit are not to be taken as applying to other animals, owing to the peculiar aptitude of this animal to intra-vascular clotting.

With extremely rare exceptions, in which intravascular clotting may occur, defibrinated and even hæmolysed blood from the same species are innocuous to the cat and dog.

If the blood-pressure is caused to fall by any means there may be reduction of renal secretion, but this is restored by the intravenous injection of gum-saline.

Hæmoglobin dissolved in the plasma is able to serve as a carrier of oxygen, but the experiments do not enable a statement to be made as to whether it is as efficient in this respect as the red corpuscles are.

It appears that the serious results of the transfusion of incompatible blood are not to be ascribed to the hæmolysis as such, but are rather an aspect of the action of foreign serum protein analogous to that responsible for anaphylactic shock.

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THE DETERMINATION OF THE REACTION OF BACTERIOLOGICAL CULTURE MEDIA.

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INTRODUCTION.

The importance of a correct adjustment of the reaction of bacteriological media cannot be over-estimated if one wishes to obtain uniform growths or constant toxin production. The truth of this has been very clearly demonstrated in recent work on anaërobic bacteria.

In the ordinary course of bacteriological work one comes across from time to time a batch of media which, for some reason or another, fails to give satisfactory growths. Yet the individual constituents of the defective medium may be of the same quality as the good medium. In such cases it is not uncommon to find that the "reaction" of the medium is at fault, usually on the acid side. It is generally accepted that the reaction of the

medium—when dealing with pathogenic bacteria—should correspond to that of the tissues. Atkin (1), using what he termed the neutral point of phenolphthalein (first perceptible darkening without any change of colour), showed that a common optimum of growth is obtained with broth of this reaction. In actual practice the production of a medium of a particular reaction is attended with considerable difficulty, and a good deal of work has been carried out to improve the technique of standardisation. Formerly the only test applied was litmus paper: a blue coloration was considered to indicate a satisfactory reaction. The crudeness of this method soon became apparent when one was dealing with the more delicate organisms, and accordingly measures were taken to remedy the defect. Schultz (2) appears to have been the first to advocate actual titration. His work was supplemented and improved by Fuller (3) in America, and Eyre (4, 5) in this country. The principle employed was to estimate by titration with N/10 soda the degree of acidity, using phenolphthalein as an indicator. The end-point of phenolphthalein is somewhat too alkaline for use, and therefore N/1 HCl was added to the "neutral" broth and the reaction was described as being, say, 10 c.c. N/1 HCl per litre, or shortly "+ 10."

Since the above methods were devised, however, great advances have been made in our knowledge of the "reaction" of fluids. Acidity means excess of H- over OH-ions, and alkalinity the reverse. Neutrality is merely the presence of equal numbers of H- and OH-ions per c.c. The purest re-distilled water is taken as the standard of absolute neutrality. According to Sörensen's scale, which is explained later in this paper, its value is P_H 7·07. Normal blood lies slightly on the alkaline side of this neutral point.

It is important in all investigations which deal with the standardisation of biological methods that a simple but sound system of nomenclature and symbols should be used. The exponential method, first suggested by Sörensen, is usually admitted to satisfy most requirements. The requisite amount of mathematical knowledge required is slight. It is hoped that the following remarks with reference to the ionic theory and the law of mass action will make clear the notation adopted.

The ionic theory was first propounded by Michael Faraday in 1833, and since then greatly extended by the work of Clausius (1857), Arrhenius (1887), Kohlrausch and many others. The theory was intended to furnish an intelligent explanation why some solutions were conductors of electricity and others not. Pure water scarcely conducts electricity at all, a solution of $CuSO_4$ extremely well, while it is believed that if water could be obtained absolutely pure it would be a non-conductor of an electric current. Faraday suggested that the electric current is carried by the dissolved matter in the solution. The current is led into the solution by a plate called the "anode," and conducted out by another plate, the "kathode." Now it is known that the anode acquires a positive electrical charge and the kathode a negative one.

Faraday suggested the dissolved salt $CuSO_4$ was split into two component parts, oppositely and equally charged with electricity, the Cu positively and the SO_4 negatively. This splitting up into two parts he called "ionisation." Evidently now the positive ions will be attracted to the kathode and the negative ions to the anode. The charge on each ion is neutralised by the electrodes, and so in each case the latter have to draw upon the source of electrical power for more electricity. This, therefore, constitutes the electric current. It has further to be supposed that the constituent ions have temporarily lost all chemical individuality, and that when they have had their charge neutralised they regain their chemical characteristics, and can be recognised as such, *e. g.* Cu by the familiar coppery deposit on the kathode, while the SO_4 attacks the water around the anode, unites with its hydrogen, regenerates H_2SO_4 and leaves behind un-ionised oxygen, which is found collected as bubbles on the anode. As water is slightly conducting one must suppose it is slightly ionised and so contains H- and OH-ions. Briefly, then, all conducting solutions are ionised. The ions are equally and oppositely charged, and in this condition their chemical properties are hidden.

The "reaction" of a medium.—From what has been said it will be seen that in an acid medium containing, say, HCl there must be H-ions and Cl-ions. Further, all acid media contain H-ions, and this constitutes the distinctive feature of any "acid" medium. Similarly any alkaline medium such as caustic soda NaOH ionises into Na- and OH-ions. In all alkaline media there exists an excess of OH-ions—again a distinctive feature. If both OH- and H-ions exist together in a solution, then the "reaction" would be determined by the nature of the majority, *e. g.* if H-ions are in excess then it is "acid," while if the OH-ions are in the majority it is "alkaline." Thus a "neutral" medium would be one in which there is an equal number of H- and OH-ions per c.c. It should be pointed out that the H-ions may come from several sources—inorganic acid, organic acid, or acid salt (*e. g.* $KHSO_4$).

Different acids, however, vary greatly in their relative strengths, depending upon their degree of ionisation. Thus HCl is highly ionised and thus is a "strong" acid, while acetic acid is "weak," because it is relatively slightly ionised. In like manner the degree of alkalinity depends on the extent of ionisation into hydroxyl- (OH-) ions. The comparative strengths of different acids is best expressed by means of their dissociation constants. In order to explain this we must refer to the Law of Mass Action.

Mass action.—Suppose two molecules A and B react to produce two other molecules C and D, then in many instances it is known that the action does not proceed indefinitely. When a certain percentage of A and B has been converted into C and D the reaction ceases. The amount changed depends on a variety of circumstances. Given a definite set of conditions then the percentage of A converted into C and D is definite and constant. This was expressed by Guldberg and Waage in 1867 in the form of a "law."

"The velocity of a reaction is proportional to the concentration of the reacting substances," *e. g.* if A is acetic acid and B is ethyl alcohol, then C is ethyl acetate and D is water. The reaction is written thus:

$$CH_3 . COOH + C_2H_5 . OH \rightleftarrows CH_3 . COOC_2H_5 + H_2O.$$

In this case two-thirds of the acid are converted and no more. As a simpler illustration we may quote $H_2O \rightleftarrows H + OH$. This law is capable of mathematical expression, and we direct attention to this in order to show the meaning of the symbol "P_H," or, as it is sometimes written, "pH." Let the concentrations (*i. e.* mass per unit volume) of A, B, C, D be represented by (A), (B), (C), (D). Also let V_{AB} represent the "velocity" of the reaction between A and B to produce C and D, *i. e.* mass converted per unit time. Similarly V_{CD} denotes the rate of conversion of C and D into A and B.

Therefore—

$$V_{AB} \propto (A)\ (B),\ i.e.\ V_{AB} = \lambda\ (A)\ (B),$$
$$V_{CD} \propto (C)\ (D),\ i.e.\ V_{CD} = \mu\ (C)\ (D),$$

λ and μ being constants.

When the reaction is at an end, the velocity of change of A and B into C and D must, by the kinetic theory, equal that of C and D into A and B.

So ultimately $V_{AB} = V_{CD}$, *i. e.* $\lambda\ (A)\ (B) = \mu\ (C)\ (D)$.

Therefore $(A)\ (B)\ (C)\ (D) =$ a constant.

Applying this to the case which concerns us most, viz. $H_2O \rightleftarrows H + OH$ we have—

$$\lambda\ (H)\ (OH) = \mu\ (H_2O) \text{ or } (H)\ (OH) = k\ (H_2O),$$

where k is a constant.

Now the concentration of H_2O molecules is great and is relatively unaltered. It is usual to regard it as constant, and so we may write $(H)\ (OH) = k\ (H_2O) = K$, which is called the "dissociation constant."

The value of K has been found by various means to be approximately $10^{-14\cdot14}$ at 18° C. Now, in a perfectly neutral solution (*i. e.* absolutely pure water) there must obviously be as many H- as OH-ions. It therefore follows that $(H) = (OH)$, and so $(H) . (OH) = (H)^2$, *i. e.* $(H)^2 = 10^{-14\cdot14}$, and $(H) =$ the square root of $10^{-14\cdot14} = 10^{-7\cdot07}$. In an acid solution the (H) must be greater than $10^{-7\cdot07}$, of course in an algebraic sense, while the (OH) is less than $10^{-7\cdot07}$, the product of the two always being $10^{-14\cdot14}$. It will also be seen that $(H) = K/(OH)$ or $(H) = 10^{-14\cdot14}/(OH)$ and $(OH) = 10^{-14\cdot14}/(H)$.

For purposes of measurement or the tabulation of results it is only necessary to deal with hydrogen-ion concentrations. The OH values can always be deduced from them if required.

The values quoted above for hydrogen-ion concentrations are always negative powers of 10. In all calculations one would naturally use ordinary logarithms. Sörensen (6) suggested that these indices (with the negative sign dropped) should be used to designate the ionic concentrations of the solutions

being dealt with. This saves a great deal of space and is exceedingly convenient. To the non-mathematical reader, however, it presents some difficulty.

The symbol chosen for this index of the hydrogen-ion concentration (with negative sign understood) is P_H. A few examples may make the idea clear:—

The H-ion concentration of pure water has been shown to be $10^{-7\cdot07}$. Thus its P_H is 7·07.

Again, N/100 HCl solution should contain ·01 grm. of hydrogen in a litre. This is only true if all the hydrogen were present as ions. Ostwald, however, showed that ionisation varied with the degree of dilution. It has been found by various means that at room temperature only 96 per cent. of the hydrogen is ionised. So the H-ion concentration of N/100 HCl is 96 per cent. of 10^{-2}, *i. e.* ·0096. Now the "logarithm" of ·0096 is $-3+0\cdot98$, which is written $\bar{3}\cdot98$, but it is easier to use the negative index $^{-2\cdot02}$. So the H-ion concentration of N/100 HCl is $10^{-2\cdot02}$ or $P_H = 2\cdot02$ if the negative sign is understood.

Further, N/10 NaOH has a H-ion concentration of $10^{-14\cdot14}/10^{-1}$, *i. e.* $10^{-13\cdot14}$. But only 84 per cent. is ionised. So this alters the value to $10^{-14\cdot14}/_{\frac{84}{100}}$ of 10^{-1}, which is very approximately $10^{-13\cdot06}$. Hence P_H of N/10 NaOH is 13·06.

Buffer solutions.—It is obvious that the addition of even a minute quantity of well-ionised acid or base must easily alter the P_H value of water. But if one uses other solutions instead of water, then the above generality does not always hold. For example, a little HCl added to a sodium acetate solution produces surprisingly little alteration in the P_H value. On investigation it is found that the cause is the fact that the acetic acid produced ionises with difficulty. That is, the H-ions are used up by the CH_3COO and so the mixture does not show much increase in P_H. Such solutions are called "buffer solutions." Other examples are phosphates, citrates, borates, proteins and amino-acids.

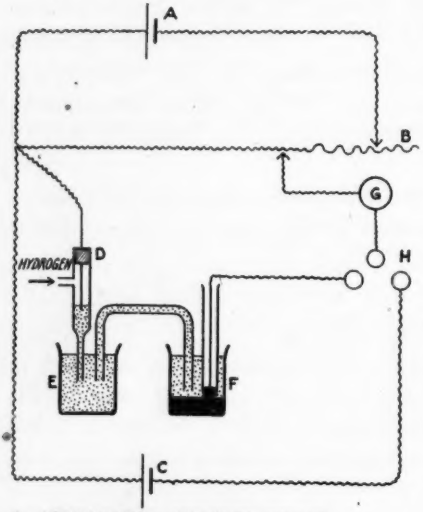
Chemical titration.—In ordinary titration by chemical means one assumes that x c.c. of N/10 acid are neutralised by x c.c. of N/10 alkali. This is so as regards total effect, but it is no measure of the "acidity" or "alkalinity" of the solutions used. In the end, the total amount of acid and alkali is neutralised, but it gives no idea of the scarcity or abundance of ions present. During the process HCl is freely ionised and so its neutralisation is accomplished "*en bloc*" as one might say. On the other hand, if the acid were CH_3COOH the ionisation is very poor. In this case neutralisation proceeds in instalments. Or it might be expressed thus: as fast as the ions are neutralised, fresh undissociated acetic acid molecules proceed to break up, giving more H-ions, in an effort to keep the H-ion concentration of the

solution constant. This goes on till all the acid present has been completely dissociated. It is this concentration of ions which is so important in considering the reaction of bacteriological media, and therefore it is only by going back to a truly fundamental method of measurement that one is enabled to estimate the character of a medium.

Therefore we propose the correlation of Eyre's scale of acidity, obtained by chemical titration, with a P_H scale. This is developed in greater detail further on in the paper.

ESTIMATION OF THE HYDROGEN-ION CONCENTRATION.

In the estimation of the hydrogen-ion concentration of different fluids two methods are commonly used — (*a*) the hydrogen electrode, (*b*) the colorimetric.



A. ACCUMULATOR FOR MAIN CIRCUIT
B. ADJUSTABLE RESISTANCE
C. STANDARD WESTON CELL
D. HYDROGEN ELECTRODE
E. SATURATED KCl SOLUTION
F. CALOMEL ½ ELECTRODE
G. CAPILLARY ELECTROMETER
H. SWITCH

FIG. I.

(*a*) *The hydrogen electrode method.* —This depends on the fact that there exists a definite potential between any metal and its salts. Here we are dealing particularly with hydrogen (electrolytically a metal) and its acids (really salts of hydrogen). If, therefore, we have an electrode coated with platinum or palladium to occlude the hydrogen, and immersed in an acid or alkaline solution, it follows that a definite E.M.F. exists between this hydrogen electrode and its solution. The E.M.F. varies with the strength of the solution, *i. e.* with the H-ion concentration. To any value of the E.M.F. there corresponds a definite value of the H-ion concentration. Full details of this method are to be found in Sörensen's (6) and Michaelis' (7) articles.

This method is carried out by the use of a potentiometer and hydrogen electrode. A short description of the apparatus and technique is given:

(1) The electrode was of the "upright" type (Walpole (8)), of capacity about 1 to 2 c.c., which could be adjusted as desired.

(2) The potentiometer was kindly lent by Dr. H. H. Dale, F.R.S., of the *Medical Research Committee*, to whom we offer our best thanks for its use.

(3) The standard half-cell was of the saturated calomel type. It was made by us with great care of the purest materials.

(4) The connecting solution was saturated KCl. It was chosen because it gives the least difference of contact potential, and is the easiest to set up and maintain.

(5) The point of balance was determined by aid of a capillary electrometer.

(6) Hydrogen was generated by electrolysis of water.

The general arrangement of the apparatus was as shown in Fig. 1.

A standard cell (Weston), as shown in the figure, is also inserted into the apparatus so that the readings of the hydrogen electrode may be at once standardised. The instrument reads E.M.F. to ·001 volts. This E.M.F. is translated into H-ion concentration or P_H values by means of a table, graph, or formula.

(*b*) *Colorimetric method of estimating* P_H.—Indicators change their colours with change in P_H values. The limits of change for any one indicator are sometimes fairly sharp, while for another they may be relatively broad. As, however, there are many scores of indicators each with its own so-called "neutral" point it will be seen that it is quite possible to cover a wide range in P_H values by a suitable choice of indicators. Various devices have been used to secure forms of apparatus for determining with tolerable accuracy the P_H value of any given solution. Obviously the results are not so good as the use of the hydrogen electrode apparatus, but at any rate they are rapid, and easy to work with.

SOURCES OF ERROR IN ESTIMATING THE H-ION CONCENTRATION OF MEDIA.

We here give the routine scheme formerly used in this Laboratory for the preparation of media. In each case the basis is a watery extract of meat. From this the ordinary broth is prepared and from it the solid media.

(1) Ordinary broth: (*a*) 1 lb. of meat free from fat is minced, and then macerated in cold water (1 litre) overnight. As an alternative to this the mixture may be macerated at 40° C. for half an hour.

(*b*) Boil for ¼ hour over a gas-ring, filter through muslin and then through paper. Make up 1000 c.c. and add 10 grm. peptone (Morson or Witte) and 5 grm. salt. Dissolve and steam for 20 minutes. The medium should now be ready for standardisation.

(*c*) Measure 10 c.c. broth into a porcelain evaporating dish. Add 25 c.c. distilled water. Have a control dish alongside prepared in exactly the same manner. Add 5 drops of ½ per cent. alcoholic solution of phenolphthalein to the first dish. Warm it over gauze by a Bunsen burner. When boiling run in N/10 NaOH from a burette. Stir continuously and look for a slight but

definite darkening of the liquid. This might better be explained as the first but distinct change in tint (faint rose, or, as some call it, a salmon tint) of the fluid as compared with that of the control. No distinct pink should appear. A good test for the end-point is obtained by allowing the liquid to cool, when a definite pink shade should appear. Further, just before the end-point is reached a precipitate of phosphates occurs. Note the quantity of N/10 NaOH required to produce this. With these data a calculation is made as to the amount of N/1 soda required to produce the same reaction in the rest of the broth. This amount of soda is then added and the flask shaken.

(*d*) The neutral broth is now steamed for half-an-hour to deposit the phosphates and then filtered free from them. The reaction has now to be adjusted by the addition of 5 c.c. of N/1 HCl per litre of broth. With the above "end"-point we found that media corrected to + 5 had reactions corresponding to that of plasma P_H 7·6.

(*e*) Lastly, it is placed in tubes or flasks and sterilised by heat in the autoclave for twenty minutes at 115° C.

(2) Glucose broth is made from the above by the addition of 1 per cent. of glucose.

(3) Ordinary agar: To the ordinary broth add 2 per cent. of powdered agar. In making the agar medium the agar powder should be emulsified in a mortar with the broth and then steamed to dissolve it completely.

The above details may be followed with minute care and yet the final product is sometimes unsatisfactory as regards its reaction.

This may be due to hydrolytic changes which occur during sterilisation and lead to an increase of acidity. Or again, the process of titration may be in error quite apart from accidental errors (arithmetical calculation). The most important in this respect, as we shall show later, arises from the difficulty in judging accurately the "end-point" of the indicator selected.

Further, there is a possibility of an error in P_H arising in bringing the medium back to the reaction selected, and there is a question whether media should be titrated at the boiling-point or at room temperature.

We have attempted to analyse each of these points, and in addition we have endeavoured to devise a method whereby such errors may be eliminated or avoided, thereby yielding media which are quickly and yet accurately standardised.

Alteration of the Reaction due to Heating.

All workers on this subject have stated that under the influence of heat bacteriological media tend to become more acid in reaction. But the extent to which this occurs with different media when sterilised in the ordinary manner (by steaming or autoclaving) is not very definite. For instance, Eyre states that after three steamings for three-quarters of an hour the reaction

becomes constant in the case of meat extract. Anthony and Ekroth (9), on the other hand, found that "the state of complete hydrolysis, *i. e.* the point at which no further acidity is produced, was not reached with meat infusions (which had been previously subjected to boiling over the open flame for one to two hours) even after prolonged autoclaving at 15 lb. pressure for eight hours." Clark (10) found that a meat infusion containing $\frac{1}{2}$ per cent. K_2HPO_4 brought to a reaction of P_H 7·19 before sterilisation, changed to P_H 6·70 after sterilisation. The change in reaction was accompanied by a heavy precipitate.

In the experiments quoted below we have confined our observations to the effects of what might be regarded as the maximum amount of heat employed, under ordinary conditions, to sterilise the media. In the case of sterilisation by steaming, the effects have been restricted to three steamings of 20 minutes each. The same batch of media was also autoclaved at 115° C. for 20 minutes. In some instances the media were autoclaved on two more occasions, since in preparing media it is at times necessary to re-autoclave.

Comparison of the Effects of Three Successive Steamings of Ordinary Broth (for half an hour each), with Three Autoclavings of 20 Minutes each.

Steaming.

Reaction before.		Reactions after. 1st		2nd		3rd	
E.M.F.	P_H.	E.M.F.	P_H.	E.M.F.	P_H.	E.M.F.	P_H.
0·713	8·01	0·688	7·57	—	—	—	—
?		0·690	7·61	0·685	7·52	0·681	7·45
0·733	8·35	0·723	8·18	0·711	7·98	0·706	7·88
0·692	7·64	0·691	7·62	0·690	7·61	0·688	7·57
0·722	8·16	0·731	8·32	0·729	8·28	0·722	8·16
0·734	8·37	0·732	8·34	0·730	8·30	0·728	8·26
0·712	8·12	0·718	8·10	0·717	8·08	0·716	8·06
0·729	8·28	0·726	8·23	0·722	8·16	0·719	8·12
			Autoclaving.				
0·733	8·36	0·726	8·23	0·714	8·02	0·710	7·96
0·692	7·64	0·687	7·55	0·685	7·52	0·685	7·52
0·722	8·16	0·733	8·35	0·722	8·16	0·720	8·12
0·734	8·37	0·728	8·26	0·714	8·12	0·713	8·01
0·717	8·12	0·714	8·02	0·708	7·92	0·704	7·85
0·729	8·28	0·714	8·02	0·693	7·66	0·667	7·20

After three steamings the average variation in H-ion concentration is about

P_H 0·13, while after the same number of autoclavings the figure is 0·23. The total variation in reaction due to sterilisation in the case of ordinary broth is therefore very small, and not likely to interfere with bacterial growth, provided, of course, one starts with a reaction which is on the alkaline side of neutrality.

Repetition of the Experiment with 1 *per cent. Glucose Broth.*

Steaming.	E.M.F.	P_H.
Reaction before . .	·729	8·28
Reaction after (1st) .	·726	8·23
,, (2nd) .	·722	8·16
,, (3rd) .	·719	8·11

Autoclaving.	E.M.F.	P_H.
Reaction before . .	·729	8·28
Reaction after (1st) .	·713	8·00
,, (2nd) .	·693	7·66
,, (3rd) .	·666	7·19

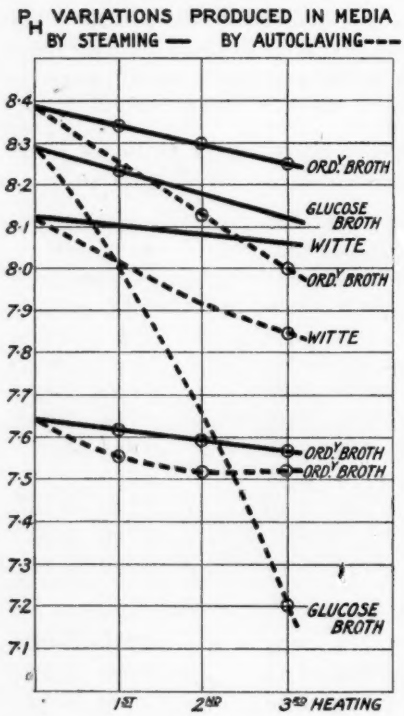


FIG. 2.

With carbohydrate-containing media, the results indicate clearly that there is a considerable tendency to the production of acid during the process of sterilisation by heat. This, of course, is no more than one would expect from the fact that hydrolysis of carbohydrate into smaller molecules proceeds more rapidly with rise of temperature or prolongation of action. In the case of glucose broth this is very well marked after autoclaving; great disruption of the molecule must occur. In fact, after an autoclaving the reaction can change by as much as P_H 1·12. Obviously, under these circumstances, sugar-containing media should never be autoclaved. Agar itself is neutral in reaction, so that the addition of it to ordinary broth will tend to lower the alkalinity of the finished product, and agar made with broth of a reaction of P_H 7·8 will probably have a reaction of P_H 7·4. Thus the final reaction of the agar should be carefully checked.

The above experimental values are plotted to show visually the difference in the effects of repeated steamings *versus* autoclavings (see Fig. 2). Ordinary broth and agar vary but little, as will be seen by either steaming or autoclaving,

but glucose is seriously decomposed, while three steamings are never so destructive as one autoclaving. The application therefore of any unnecessary heat in the preparation of bacteriological media, and especially when carbohydrates are present, is to be avoided. Failure to observe this will certainly result in upsetting the final reaction.

Difficulty of Judging the End-point of the Indicator selected.

Leaving out of account the possibility of accidental errors such as arise from measuring or calculation, we think that the most frequent source of error arises in adjudging correctly the end-point selected. To a certain extent this trouble exists with most indicators, but is more marked with some than with others. The selection of an arbitrary shade of colour, or the matching of two colours, as is the usual practice, allows of a great possibility for errors. Thus an indicator should be selected which not only changes at the reaction required, but which gives a constant and easily recognisable "end-point."

We have tried a series of indicators including rosolic acid, neutral red, litmus, cresol red, thymol blue, tetra-brom-phenolphthalein, phenolphthalein thymolphthalein, etc., in our attempts to find the best with which to titrate media.* In our hands phenol- and thymolphthalein proved the most suitable. With both of these the change is from a colourless to a coloured solution, while the change takes place within a slight range of hydrogen-ion concentration—in fact, within a half P_H with the first indicator and rather less with the second.

The objection has been raised that these two phthalein indicators change colour at a point on the alkaline side of neutrality. But in bacteriological work this is really no objection, as it is necessary during the process of preparation to bring the meat extract to an alkaline reaction in order to get rid of the excess of phosphates. If one does not do this the broth will not be clear and the phosphates will be constantly precipitating out, which, from the practical point of view, is a considerable objection.

We shall deal first with *phenolphthalein*, as it was with this indicator that the majority of the comparative tests were made. Phenolphthalein, as anyone familiar with bacteriological work knows, has been almost universally used in the titration of media; but there is a lack of uniformity as to the end-point, and a great variety of arbitrary shades of colour have been advised by different writers. The end-point we have used corresponds to the first development of a trace of pink or a salmon tint in the medium, and not to the development of a *distinct* pink colour. With the broth diluted about three times this change is fairly sharp, if one uses another dish alongside as a control, as already described. According to Clark and Lubs (11), the addition of water does not appreciably alter the H-ion concentration, and we also found that

* The indicator dyes which we have used were prepared and supplied to us by the Cooper Laboratory, Watford.

dilution did alter the reaction to a measurable extent. This end-point or "neutral point" can be judged much more exactly than any arbitrary shade of pink.

When titration is carried out at 100° C., the first distinct change of shade or development of a faint trace of pink with phenolphthalein corresponds to P_H 8·0–8·1 (tested after medium allowed to cool). The P_H reactions of the various pinks and reds used by bacteriologists are really very variable, as at that stage it requires a considerable amount of alkali to make any perceptible change in the shade of the colour. In fact this definite pink can correspond to any P_H from 8·2 to 8·7 or even 9·0.

The following results show the reaction of our "neutral point" of phenolphthalein (faintest pink or salmon) when titrated at 100° C.:

Observer.	E.M.F.	P_H.
J. G. . . .	·712 .	8·00
J. M. . . .	·723 .	8·18
,, . . .	·715 .	8·05
,, . . .	·719 .	8·11
J. S. . . .	·725 .	8·20

This gives an average of P_H 8·10.

So far our experience shows that with *thymolphthalein* even more accurate estimations can be obtained. This is due to the ease with which its "neutral point" can be judged. The neutral point is taken as the first perceptible darkening of the medium on the addition of N/10 soda. The titration is carried out exactly in the manner described for phenolphthalein, *but at room temperature.* Ten c.c. of the broth to be used is placed in a porcelain evaporating dish and 25 c.c. of distilled water added. A control dish is prepared in exactly the same manner. Add 5 drops of a ½ per cent. alcoholic solution of thymolphthalein to the first dish. Then run in N/10 NaOH from a burette. Stir continuously and look for a slight but definite darkening of the liquid. This might more aptly be described as the disappearance of the yellow tint and the development of a bluish tinge. The change in the medium occurs quite sharply, and at times a single drop of soda in excess will change the medium to a greenish-blue colour. To titrate media the average of at least three readings should always be taken, and the first reading, in which a blue colour should be sought, rejected. Two factors are probably at work in the production of this sharp end-point, namely the relative sensitivity of thymolphthalein to a slight excess of alkali after a certain ionic concentration has been reached, and secondly to the change of colour, which is from a yellowish to a blue tint.

The relative ease with which this end-point can be reached is well shown in the following results obtained by different observers. In every case 10 c.c.

of broth was diluted with 25 c.c. of distilled water, and 5 drops of 0·5 per cent. alcoholic solution of thymolphthalein added.

Sample A.

N/10 soda required.	E.M.F.	P_H.	Observer.
3·6 c.c.	·775	9·08	J. G.
3·5 c.c.	·771	9·05	,,
3·4 c.c.	·770	8·99	J. M.

Sample B.

N/10 soda.	E.M.F.	P_H.	Observer.
4·2 c.c.	·776	9·09	J. G.
4·1 c.c.	·771	9·01	,,
4·1 c.c.	·768	8·96	J. M.
3·8 c.c.	·760	8·81	,,
3·9 c.c.	·762	8·85	M. H.
3·8 c.c.	·760	8·81	,,
4·1 c.c.	·778	9·13	L. G.
3·8 c.c.	·764	8·89	,,

The average P_H worked out at 8·95. The last two observers had never used this reagent before, yet their results correspond very closely with the other findings.

The objection, that such a reagent as thymolphthalein necessitates the addition of an excess of alkali, has already been dealt with, while a series of experiments showed that the addition of 10 c.c. of N/1 HCl per litre was sufficient to correct this excess and leave the final reaction of the medium, after sterilisation, at about P_H 7·6. (Fig. 4 is a graph by which the amount of acid necessary to bring the final reaction of the medium to any desired reaction can be found.)

Titration at the Boiling-point or at Room Temperature.

According to routine technique, titration with N/10 NaOH is performed at the boiling-point. The question arises as to whether this is justifiable and to what extent, if any, it interferes with the end-point. We have endeavoured to settle this matter by a determination of the P_H value at different temperatures. *A priori* one would easily imagine that at higher temperatures there should be greater dissociation and hence greater acidity. This was found to be the case but only when the liquid was *partially* dissociated in the cold. When substances like HCl or NaOH are tested there is no increase of acidity on heating.

Broth.	Sample	1	2	3	4	5
Hot (70° C.)	E.M.F.	·753	·748	·764	·753	·746
	P_H	8·69	8·61	8·89	8·69	8·58
Cold (room temperature)	E.M.F.	·690	·673	·730	·726	·704
	P_H	7·60	7·31	8·30	8·23	7·85
Difference	P_H	1·09	1·30	0·59	0·46	0·73

N/10 NaOH, N/10 HCl, and acetate mixture gave no appreciable difference whether hot or cold.

This explains why the broth as normally titred out becomes pink on cooling. There is less dissociation, or we may say that some of the dissociated molecules at the boiling-point have reunited to form un-ionised molecules. Obviously, then, one should titre at room temperature, or as near as possible the temperature at which the organisms are grown.

Using our neutral end-point we found that phenolphthalein did not give a sufficient degree of alkalinity when the titration was carried out in the cold. The reaction of the media in this case was rather under P_H 8·0. With thymolphthalein, however, a sufficient degree of alkalinity is easily obtained with this end-point as has already been indicated.

RELATION OF THE EYRE NOTATION TO THE HYDROGEN-ION CONCENTRATION.

After neutralisation it is necessary to bring media to a degree of alkalinity approximating to that of plasma (P_H 7·6). This is done by the addition of acid to the "neutral" broth. Eyre's method is to add 10 c.c. of N/1 HCl to every litre of broth which has been brought to his "end-point" of phenolphthalein. With our "end-point" the necessary amount of acid in the case of phenolphthalein is 5 c.c. and with thymolphthalein 10 c.c.

The addition of this acid, however, can only give a constant P_H to media of constant composition. It does not necessarily follow that if two samples of medium have a different composition the addition of an equal quantity of acid will ensure the same P_H.

The following experiments will show that there is a constant relation between Eyre's scale and P_H when the medium is constant.

Experiment I.—In the first experiment increasing amounts of N/1 HCl corresponding to 1 to 10 c.c. per litre were added to ordinary peptone broth. The reaction of the broth was neutral to phenolphthalein at 100° C. (Fig. 3).

Results as follows were obtained:

Amount of N/1 Acid added per Litre.

	0	2 c.c.	4 c.c.	5 c.c.	6 c.c.	8 c.c.	10 c.c.
E.M.F.	·737	·725	·714	·708	·704	·692	·682
P_H	8·42	8·22	8·02	7·92	7·85	7·64	7·46

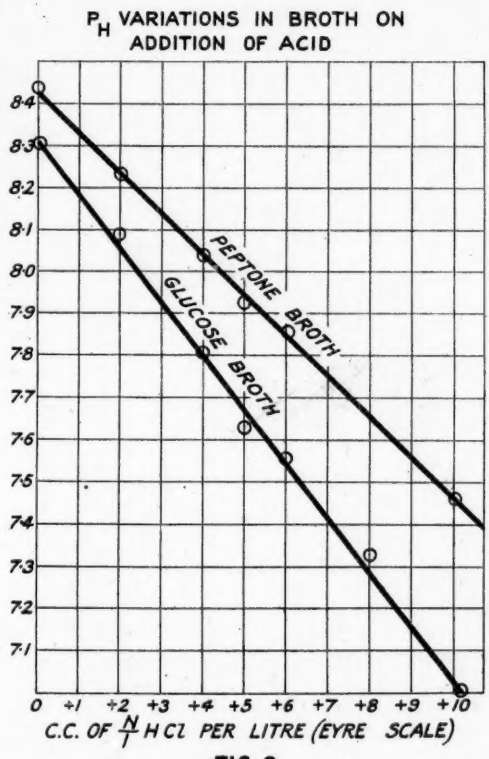


FIG. 3.

With 2 per cent. glucose broth very slightly different results were obtained:

Amount of N/1 Acid added per Litre.

	0 c.c.	2 c.c.	4 c.c.	5 c.c.	6 c.c.	8 c.c.	10 c.c.
E.M.F.	·729	·717	·701	·691	·687	·673	·654
P_H	8·28	8·08	7·80	7·62	7·55	7·32	6·98

Experiment II.—In this experiment broth which had been made neutral to thymolphthalein at 15° C. was used (Fig. 4).

Amount of N/1 Acid added per Litre.

	0 c.c.	2½ c.c.	5 c.c.	10 c.c.	15 c.c.	20 c.c.
E.M.F.	·765	·749	·739	·702	·670	·637
P_H	8·90	8·66	8·45	7·82	7·25	6·68

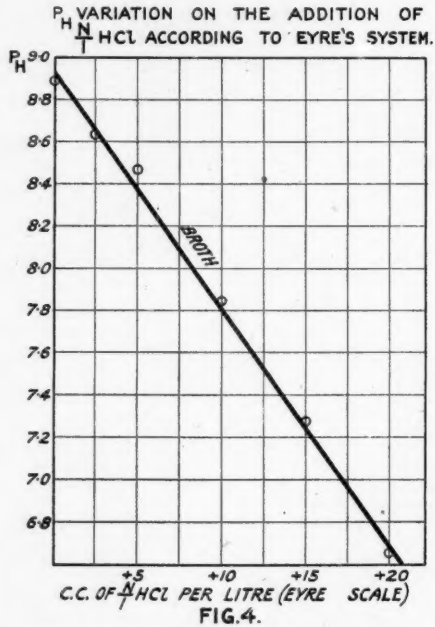


FIG. 4.

Experiment III.—In this experiment a parallel series was tested, the first made neutral to thymolphthalein and the second neutral to phenolphthalein.

Amount of N/1 Acid added per Litre of Broth Neutral to Thymolphthalein.

	0 c.c.	5 c.c.	10 c.c.	15 c.c.	20 c.c.	25 c.c.	30 c.c.
E.M.F.	·755	·731	·703	·675	·652	·623	·602
P_H	8·73	8·32	7·83	7·34	6·94	6·43	6·06

Broth Neutral to Phenolphthalein.

E.M.F.	·740	·712	·690	·662	·635	·607	·580
P_H	8·48	8·00	7·60	7·12	6·65	6·15	5·70

Averaging the results obtained with ordinary broth, prepared in the manner described in the first part of the paper, it would appear that there was a constant relation between the P_H and the amount of acid added. In fact the addition of 1 c.c. of N/1 acid per litre makes a change of 0·1 P_H in the reaction.

Working on this basis it is possible to construct a graph by which one can find the H-ion concentration of broth from the amount of acid added, provided, of course, one knows the exact H-ion concentration of the broth previous to the addition of the acid, and that the medium has been made according to formula. Fig 5 has been so constructed by averaging the results of several readings.

If one uses a broth made according to another formula the relation between the acid added and the end-reaction is upset, as experiment No. 4 shows.

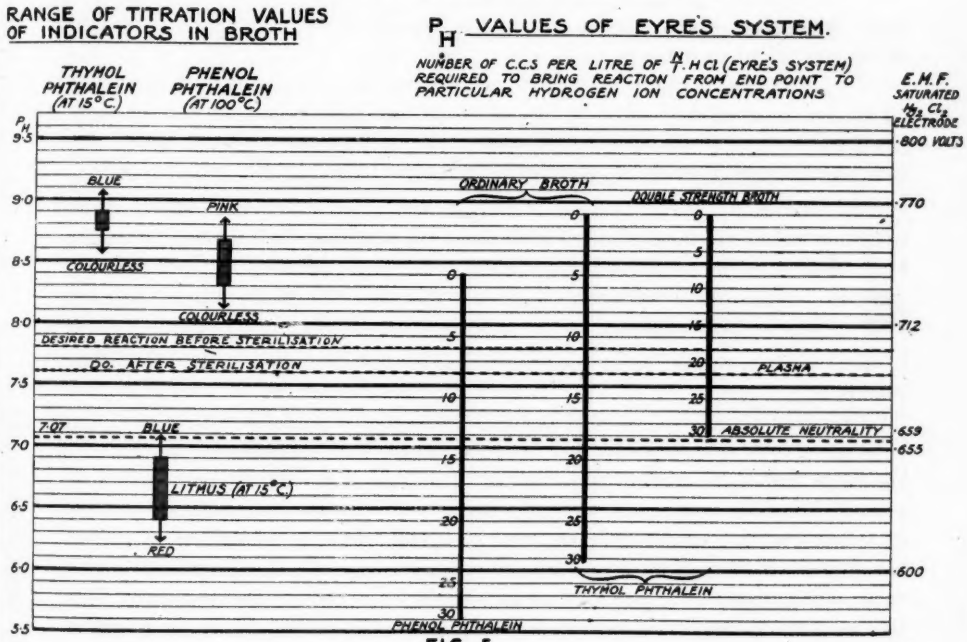


FIG. 5.

Experiment IV.—The broth on which the observations were made, was made up in double strength—that is, in making the meat extract, twice the quantity of meat was used and 2 per cent. of peptone added. The preparation of double-strength broth is frequently employed in making up our media. It is a simple way of making up nutrient agar, as the agar is first dissolved in water and then added to an equal volume of the double-strength broth.

Amount of N/1 Acid added per Litre.

	0 c.c.	5 c.c.	10 c.c.	15 c.c.	20 c.c.	25 c.c.	30 c.c.
E.M.F.	·766	·757	·742	·727	·703	·688	·665
P_H	8·92	8·76	8·51	8·25	7·83	7·57	7·18

Thus instead of 5 c.c. of N/1 acid per litre making a difference of 0·5 P_H, it makes a difference of 0·3 P_H.

These results are of great practical value, as it means that the older chemical titration of media in conjunction with a graph can give an accurate indication of the H-ion concentration, and so overcome one of the chief objections to the use of the purely chemical method. It is, however, necessary that the media be prepared in a constant manner and the initial reaction known. We have found that the P_H values obtained by graphs from the Eyre system are sufficiently reliable for the standardisation of media, especially when controlled in bulk.

To check the reaction in bulk it is not necessary to titrate out the broth again, but only to show that it lies between two points. This can be done most conveniently by a suitable choice of indicators. Theoretically, the indicator points should lie as near as possible to P_H 7·60, but the lack of suitable indicators prevents this. Comparison against a known colour shade might be used, but the technical difficulties in preparing the necessary solutions and their non-permanence make them almost impracticable for ordinary routine. As a quick and reliable procedure one cannot do better than use two indicators with reaction points sufficiently near P_H 7·60. After a series of tests we have found that cresol-red and phenolphthalein give satisfactory results.

In order to carry out this control two small samples (3 c.c.) of the medium are diluted in test-tubes with an equal bulk of distilled water. To the first tube are added 2 drops of 0·5 per cent. alcoholic solution of phenolphthalein, and to the second 2 drops of 0·02 per cent. watery solution of cresol-red to which 15 per cent. alcohol may be added as a preservative. If the reaction is correct, no colour change should occur in the first tube, while a rose to a pinkish colour should be noted in the second. Thus the reaction of the tested sample will not be as high as P_H 7·90, the point at which phenolphthalein first shows a trace of pink, but will be over 7·6, cresol-red developing a rose-pink (weak permanganate colour) at P_H 7·60. If this rose colour does not appear, but only a brown shade, the medium is on the acid side, while if a pink is obtained with phenolphthalein the medium is too alkaline. The error in either direction can be adjusted by the addition of a few drops of N/1 NaOH or HCl to the medium in bulk and the reaction again controlled.

Before use it is, of course, necessary to sterilise the medium by heat (autoclaving or steaming), but the increase in acidity at this stage is slight (less than P_H 0·2), and is sufficiently allowed for. The above procedure we have found quite satisfactory as an ordinary routine.

PROCEDURE RECOMMENDED FOR THE PREPARATION AND STANDARDISATION OF ORDINARY BROTH.

(1) Mince 1 lb. of meat free from fat; add 1000 c.c. of cold water. Boil for ½ hour over a gas ring, filter through muslin and then through paper.

(2) Emulsify 10 grm. of peptone (any standard peptone such as Morson's or Witte's) in a mortar with some of the meat water. Then add the peptone together with 5 grm. of salt to the bulk of meat water.

(3) Steam for 20 minutes to dissolve the peptone completely; make up to 1000 c.c. The medium is now ready for standardisation.

(4) Measure 10 c.c. of the broth into a porcelain evaporating dish; add 25 c.c. of pure distilled water. Have a control dish alongside prepared in exactly the same manner. Add 5 drops of ½ per cent. alcoholic solution of thymolphthalein to the first dish. Then run in N/10 NaOH from a burette, stirring continuously, and looking for a darkening of the colour as compared with that of the control dish. Just before this point a precipitate of phosphates occurs. The point may be more aptly described as the disappearance of the yellow tint and the development of a bluish. Note the quantity of N/10 soda required to produce this change. In practice it is best to make four estimations, and the first in which a distinct blue has been produced is rejected. With the average data a calculation is made as to the amount of N/1 soda necessary to produce the same reaction in the rest of the broth. This amount of soda is then added and the flask shaken.

(5) The alkaline broth is now brought to the boil to deposit the phosphates, and then filtered free from them. The reaction is now adjusted by the addition of 10 c.c. of N/1 HCl per litre of broth. This will give a final reaction corresponding to that of plasma, or P_H 7·60.

(6) Control reaction with cresol-red and phenolphthalein.

(7) Lastly, place in tubes or flasks and sterilise in the autoclave for 20 minutes at 115° C.

Double-strength broth.—The double-strength broth indicated on the chart is prepared, except that all ingredients (meat, peptone, salt) are doubled, in a similar manner to that described above. Before use the medium is diluted with an equal volume of distilled water, tubed and sterilised.

DISCUSSION.

The aim of most recent workers upon the standardisation of media has been to provide a simple method which is well adapted for the routine use of a laboratory attendant. The hydrogen electrode is not, in our opinion, so adapted, and thus there has been a tendency towards colour indicators of H-ion concentration.

We ourselves find it most convenient to direct the attendant to titre out small samples of the medium with the help of a colour indicator, and to render

the whole bulk "neutral" to our indicator by the addition of the calculated quantity of alkali. Having reached this point, he can add acid as indicated on a special "graph" for the medium in use to furnish a medium of any desired P_H. Finally he checks the reaction by the use of other dyes which indicate at different H-ion concentrations.

Other workers have developed practically the use of colour scales. These scales are prepared by the addition of the colour indicator to phosphate or phthalate solutions of which the H-ion concentration is known. These solutions, however, are much more difficult to prepare than is usually supposed, and to get them accurate they must be standardised against the hydrogen electrode.

To overcome this difficulty the Medical Research Committee have arranged for Messrs. Baird & Tatlock, 14, Cross St., Hatton Garden, London, E.C., to supply standard solutions of exact H-ion concentration (12). But even allowing that the standard solutions are of the correct concentration, there is no assurance that the same shade of colour will be given in the media as the standard solutions even when their reactions are identical, as it is well known that the presence of certain salts and albuminous materials can upset to a considerable extent the normal reactions of the indicator; in fact certain indicators (litmus, etc.) may be rendered almost useless. The colorimetric method recommended by Walpole (8), Cole and Onslow (13) and others only makes adjustment for the natural tint of the medium to be tested and not for the above phenomenon. Lastly, the non-permanence of the colour of the standard is a great objection.

Quite recently it has been claimed that very exact evaluations of the reaction of media can be made without the use of the elaborate solutions of standard hydrogen-ion concentrations. This method was recently introduced by Barnett and Chapman (14), and is based on the work of Salin. It consists in superimposing the extreme colours of a particular indicator. All that is necessary is weak acid and a weak alkaline solution. Barnett and Chapman advocated the use of phenol-red, which gives a range of reactions between P_H 7 and 8.

The test is carried out as follows: Two rows of six test-tubes are placed in a double rack, and into the tubes in the front row are measured 5 c.c. of dilute acid (1 drop of concentrated HCl in 100 c.c. of water) and into the other row 5 c.c. of dilute alkali (N/20 NaOH). A solution of the indicator having been prepared (phenol-red 0·01 per cent. watery solution), drops of it are added to the various test-tubes in the amounts indicated below.

Drops per Tube.

Series no.	Front row (acid series).	Back row (alkaline series).	P_H values.
1	9	1	6·9
2	8	2	7·2
3	7	3	7·5
4	6	4	7·7
5	5	5	7·9
6	4	6	8·1

To test media with this colour scale 1 c.c. is placed in a tube and diluted with 4 c.c. of distilled water. Then 10 drops of the indicator solution are added and the shade of colour compared with that of the above couples when viewed together. The reaction of the medium can be corrected by addition of weak acid or alkali, and a simple calculation will then give the amount necessary per litre. Although this procedure overcomes the difficulty of preparing complicated standard solutions, we have not found the method as accurate as has been stated. In the first place there is the old difficulty of matching shades; secondly, the scale colours do not completely match those of the colours in broth; and thirdly, the scale is not permanent. The reaction obtained was never nearer than 0·2 P_H as found by the hydrogen electrode when the scale was freshly made.

CONCLUSIONS.

(1) For ordinary routine purposes we recommend that the adjustment of the reaction of bacteriological media is best carried out by estimating the reaction by titration of a small volume, say 10 c.c., of the medium and calculating from the result obtained the alkali required to be added to the remainder. The final reaction is adjusted by the addition of N/1 HCl.

(2) For titration, thymolphthalein was found to be the most suitable indicator. This indicator changes colour at a point on the alkaline side of neutrality which ensures that sufficient phosphates will be thrown down to prevent their precipitation in the prepared medium.

(3) Very accurate and constant readings can be determined by means of thymolphthalein, as one is not dealing with the perception of a particular shade or colour, but with a definite and distinct change in the appearance of the medium.

(4) The most suitable end-point with thymolphthalein is the so-called neutral point. This corresponds to the first perceptible darkening observed with the reagent on the addition of alkali, the titration being carried out in the cold.

(5) The reaction of all bacteriological media should be expressed in hydrogen-ion concentration, preferably in Sörensen's nomenclature.

(6) For media we have adopted a reaction corresponding to that of plasma, namely P_H 7·6.

(7) All media should be titrated at room temperature.

(8) The final reaction of any particular batch of medium should always be controlled before it is issued for use.

(9) This control is most easily accomplished by the addition of certain colour indicators to small samples of the medium. In most instances the use of two indicators will give an estimation sufficiently accurate for ordinary use. In our hands cresol-red and phenolphthalein have proved sufficient.

(10) Three steamings for half an hour are never so destructive as one autoclaving at 115° C.

(11) Glucose and other sugar-containing media are especially easily hydrolysed and should never be autoclaved, only steamed.

(12) With certain reservations the reaction of media titrated by the chemical method can by means of a graph be expressed in H-ion concentration. Thus media of any required P_H values can readily be prepared.

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ON SYMPATHETIC FEVER AND HYPERPYREXIAL HEAT-STROKE.

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In the course of investigations on the function of the thyroid-adrenal apparatus as a humoral mechanism for the heat regulation of the body, observations have been made on the experimental fever produced by the injection of β-tetrahydronaphthylamine, which for the sake of brevity will be called in this paper, "T.H.N." The object of the present paper is to point out the similarity which exists between the experimental hyperpyrexia produced by T.H.N. and hyperpyrexial heat-stroke as it occurs in man.

The observations on T.H.N. fever were made mostly on mice and rats; some were made also on rabbits. There is an intense stimulation of the activity of the thyroid and adrenal glands which is demonstrable histologically. The effect has been described and illustrated in the recently published *Sixth Scientific Report of the Imperial Cancer Research Fund* (1), and need be referred to here only briefly. Adrenin is secreted into the blood-stream and there is a general stimulation of the sympathetic system. In fact, a rabbit under the influence of T.H.N. presents an external appearance almost identical with that of an unanæsthetised animal which has received a large dose of adrenin, the chief difference being that the effect of adrenin passes off in a few minutes and does not produce a pyrexia, while that of T.H.N. is prolonged. In both cases the blood-vessels of the ear are contracted, so that the ears feel cold; the palpebral fissure is widened and there is distinct proptosis, the pupil is dilated, although sometimes the dilatation is only slight, the respiration is rapid and forcible. In T.H.N. fever the anal sphincter is relaxed, so that the thermometer does not meet with any resistance when introduced into the rectum. Occasionally there is bleeding from the rectum. These appearances are somewhat different from those seen in an anæsthetised animal after an intravenous injection of a large dose of adrenin. In such an animal there is no obvious effect on the respiration and the widening of the palpebral fissure is not so obvious as the wide dilatation of the pupil. In rabbits a very characteristic effect of T.H.N.

is a general fine muscular tremor not unlike that of a rigor, and in addition occasional "stamping" of the hind legs due to a short forcible contraction of the leg-muscles. In mice and rats the most obvious effect of T.H.N. is the forcible and rapid respiration, the marked proptosis due to the widening of the palpebral fissure and the relaxation of the sphincter. The animals are excited and the fur is ruffled. The kymograph experiments of Jonescu (6) on anæsthetised animals refer to the immediate action of the substance itself, and show a stimulation of the sympathetic system partly by a central and partly by a peripheral action. This direct effect passes off quickly, and must not be confused with the later indirect effects which set in as the result of the prolonged activity of the suprarenal gland. Observations on metabolism (14) have shown a considerable and prolonged increase in the N-excretion, which is very marked on the day of administration and persists for the next two days when the temperature has become normal and then gradually falls again. It differs in this respect as well as in its general behaviour from the over-heated animal the temperature of which has been raised by the external application of heat. In such an animal there is no effect on the N-excretion on the day of the experiment; it rises the following day, falls rapidly and reaches the normal level again on the third day. There is no glycosuria in T.H.N. fever.

Of considerable interest in connection with the premonitory symptoms of hyperpyrexial heat-stroke in man are the effects on man of small doses of T.H.N. (14). All the doses given were so small that they did not produce a rise of temperature. Doses of 0·075 grm. to 0·1 grm. produced drowsiness, giddiness, headache, palpitations and a slight feeling of oppression. Sometimes the pupils were dilated, but not so much and not so frequently as in animals, and they continued to react to light. Slightly larger doses (0·15 grm. to 0·25 grm.) produced vomiting, violent headache, giddiness, and sometimes difficulty of breathing.

The rise in temperature begins as a rule within an hour after the subcutaneous injection of a suitable dose of T.H.N., and, in rats especially, is sometimes preceded by a fall in temperature. After the first hour the temperature continues to rise rapidly for the next two or three hours. Either the animal dies in hyperpyrexia, frequently in convulsions, or the temperature falls again rapidly in the fourth or fifth hour after the injection to the normal level. If the animal dies, rigor sets in within a few minutes. The fever is due, as Stern has shown (14), to increased heat-production and to diminished heat-loss. The mode of action by which this fever is produced has already been discussed in previous publications (1), and need only be summarised here. As a result of the increased activity of the thyroid and adrenal glands there is a mobilisation of the liver glycogen. In the absence of glycogen, carbohydrate is formed from proteins and possibly also from fats. It has been shown that the body reacts to the resulting influx of carbohydrates into

the blood-stream under these conditions by an increased oxidation. Heat-production is thus increased. At the same time, heat-loss is diminished owing to the constriction of the cutaneous vessels. Since this type of fever can thus be explained as being produced by a stimulation of the sympathetic system, it may be suitably called "sympathetic fever."

The post-mortem examination of an animal which has died in T.H.N. hyperpyrexia shows as the most frequent and obvious feature an intense congestion of the lungs, which may be dark purple. Sometimes small hæmorrhages into the lungs occur which can be seen with the naked eye. The right ventricle is flaccid, the left ventricle is contracted. The trachea is often filled with mucus, the veins are engorged with dark blood. The thyroid and adrenal are deeply congested and so are the meninges. The appearance of the lungs again recalls the effect of adrenin which in toxic doses produces in unanæsthetised animals the same effect, and, in fact, often kills by hæmorrhage into the lung.

The production of hyperpyrexia by the injection of T.H.N. is dependent not only on the dose administered but also on some other factors, of which one is important for the purpose of the present paper. This factor is the climatic condition of the environment, in which the animal is kept after the injection, and the facilities it affords for cooling the animal. This can be demonstrated most clearly in the mouse, as the following experiments illustrate. Four mice were injected subcutaneously with 2·5 mgrm. T.H.N. Two mice were kept in a wooden cage with saw-dust, covered with a perforated lid in a warm room near the gas stove. The temperature inside the wooden cage was 30° C. The other two mice were kept in another room, temperature 16°, in glass jars without saw-dust, so that the mice were in contact with the relatively cold glass. Both the latter mice survived, and apart from showing a ruffled coat were not obviously affected by the T.H.N. The other two mice kept in the warm room passed into convulsions at the end of the first hour and died, the one at the end of the first hour showing post-mortem extensive hæmorrhages into the lung; the other mouse was found dead at the end of the second hour, the lungs also showing intense congestion. The weights and temperatures were as follows:

	Weight.	Before injection.	1 hour after.	2 hours after.
Mouse No. 1, hot room	17 grm.	35·1°	39·9°	Dead.
,, ,, 2, ,, ,,	22 grm.	36·4°	Dead 38·8°*	—
,, ,, 3, cool room	17·5 grm.	34·2°	35·5°	below 32°
,, ,, 4, ,, ,,	20·5 grm.	35·2°	33·4°	below 32°

* Taken immediately after death.

The importance of the condition of effective cooling in preventing or determining the onset of hyperpyrexia after the injection of T.H.N. can also be demonstrated in the following manner: A dose of 2·5–3 mgrm. T.H.N. is,

as a rule, just sufficient to produce a fatal hyperpyrexia in mice of 15–20 grm. weight if kept in a warm room in separate cages. A dose of 0·5 or even 1 mgrm. produces, as a rule, no visible effect under these conditions. But if twelve mice which have received such a small dose are kept together in a cage many of them die within the first two hours with the typical symptoms of T.H.N. hyperpyrexia. The wooden cages which are used in this laboratory are of such dimensions that ten to twelve mice can be kept together in them comfortably, and that is, in fact, the way in which mice are kept in this laboratory for weeks or months. But when there occurs in the mice a stimulation of the sympathetic system, so slight that it produces neither visible symptoms nor a rise in temperature as long as each animal is kept in a separate cage and has thus good facilities for cooling, then the climatic conditions of the crowded cage elicit a fatal hyperpyrexia. To give an example of such an experiment: after an injection of 1 mgrm. T.H.N. six out of twelve mice died in one experiment. In another experiment in which four out of twelve mice died, the temperature of the crowded cage when the first mouse died was found to be 27° C.; in the control cage with two mice the temperature was 20°. The temperatures of four mice taken from the crowded cage two hours after the injection of T.H.N. were 42·6°, 42·0°, 40·8°, 39·5°. The temperatures of four isolated mice from control cages were 38·7°, 37·8°, 37·0°, 36·9°. Two isolated mice which had received double the dose of T.H.N. and survived, without having shown very marked symptoms at any time, reached 38·4° and 38·8°.

In rats the influence of cooling was demonstrated as follows: A rat, the coat of which had been clipped, was kept together with a normal control rat of the same age and weight in a cold room. After two days both rats received 0·02 grm. T.H.N. The temperature of the normal control rat, which before the injection was 37·1° C., rose to 40·0° C. in the first two hours; that of the clipped rat remained within half a degree of the temperature of 36·4° C. which it had before the injection.

Another interesting illustration of the relation between the temperature of the environment and the hyperpyrexia produced by sympathetic stimulation was obtained accidentally in the course of an experiment, the real object of which had been to study the effect of continued stimulation of the thyroid-adrenal apparatus by repeated injections of T.H.N. A rabbit, weighing 2400 grm., received during the last half of July, 1916, four injections of 0·06 grm. T.H.N. and five injections of 0·08 grm. T.H.N. on nine different days. On only two occasions did it show a slight pyrexia, the temperature rising from the normal temperature of 39·5° to a temperature of 40·7° and 40·8° respectively. It received the last of these injections on July 28th (0·08 grm. T.H.N.), but no pyrexia resulted. After three days it was again injected, on August 1st, with 0·07 grm. T.H.N., and died in three hours with a temperature of over 44°. At the time the importance of the outside temperature had not

been recognised, and this result was looked upon as an unfortunate accident which could not be explained. On going through my protocols recently it occurred to me to consult the official meteorological records. They show that July, 1916, was an exceptionally cool month. From July 11th, 1916, to July 26th the maximal temperature ranged between 61° F. and 76° F. On July 27th the weather improved and the temperature rose to 78° F., and hot weather set in during the last two days of July and the first day of August. The connection between this change in the weather and the effect of T.H.N. appears from the following table:

Date.	Official max. temp.	Injection of T.H.N.	Result.
26/7/'16	73° F.	0·08 grm.	No fever.
27/7/'16	78° F.	,,	,,
28/7/'16	78° F.	,,	,,
29/7/'16	79° F.	No injection	—
30/7/'16	82° F.	,,	—
31/7/'16	84° F.	,,	—
1/8/'16	83° F.	0·07 grm.	Dead in 3 hours. 44°.

It may be added that the interval of three days without injections cannot be made responsible for this result, since in the preceding period such intervals had occurred; the resumption of the injections had not always resulted in fever.

Another point which may be mentioned is, that in this experiment the characteristic muscular contractions described above as "stamping," and the other symptoms of sympathetic stimulation (widening of the palpebral fissure, contractions of the blood-vessels of the ear), appeared after the injection of T.H.N. even when there was no fever. This was also observed in experiments in other rabbits. These effects are not, therefore, the results of a high body temperature acting as a stimulus to nerve or muscle.

It should be noted that besides this factor of outside temperature and of cooling, there are yet other less well understood conditions which determine the onset of sympathetic fever after the administration of T.H.N. in animals, especially in rabbits. Many rabbits fail to give any rise of temperature after doses which will kill other rabbits in hyperpyrexia, although both are kept in the same room. On going into this curious fact, it was found that rabbits which had been kept for two months or more in the laboratory, and which were well fed, sleek, and in good condition, never failed to respond with fever to a dose of 0·04 grm. T.H.N. per kilo. But the same dose would not produce any effect on rabbits which had just been brought in and which were in a much poorer condition. In rats, intercurrent diseases, such as pneumonia or a septic bronchiectasis, frequently interfered with the typical response to the administration of T.H.N. Animals so affected died, as a rule,

after the injection of T.H.N., but without having developed a rise in temperature. Sometimes, however, the animals do not die, nor do they show a rise in temperature, and no obvious cause for the failure to respond to T.H.N. could be found.

From the experiments detailed above on the importance of the outside temperature and the condition of cooling in determining the effect produced by T.H.N., it is clear that these external conditions governing the heat loss are the chief factors determining whether prolonged stimulation of the sympathetic system leads to hyperpyrexia or not. This fact may be expressed as follows: A stimulation of the sympathetic system, prolonged, but so slight that under certain conditions no obvious symptoms are produced, may be turned into a rapidly fatal hyperpyrexia by conditions which lead to an interference with the heat loss—an interference so slight that it has no ill-effect on a normal animal.

Formulated in this way the T.H.N. hyperpyrexia acquires the features of an "experimental heat-stroke" and its resemblance to hyperpyrexial heat-stroke in man becomes apparent. This resemblance extends to the post-mortem findings in hyperpyrexial heat-stroke in man and those of experimental T.H.N. heat-stroke in animals. There is early rigor, and all authors lay stress on the intense congestion of the lungs without consolidation. Manson (8) speaks of the "phenomenally engorged lungs." The venous system generally is engorged; according to the statement by Wood in Pepper's *System of Medicine* (10), "blood appears to leave the arterial system and collect in the venous trunks." Hearne (4) has found œdema and hyperæmia of the cerebral membranes, and in the brain evidence of hæmorrhages into the perivascular spaces of the larger arteries, and Rogers (13) mentions the occurrence of petechial hæmorrhages in brain, viscera and skin. All these facts agree with the postulate of the occurrence of sympathetic stimulation with the resultant high blood-pressure. Histological examination of the thyroid and adrenal glands are likely to yield further valuable and interesting information on this point. Apparently such an examination has never been carried out.

In drawing a comparison between disturbances of the heat regulation in man and in animals, it is necessary to bear in mind an important difference which exists in the physical mechanism of heat regulation. In man this is carried out largely by the activities of the sweat-glands. In most animals—the horse being one of the few exceptions—the activity of the sweat-glands is of no practical importance as a means of regulating the body temperature, since the distribution of these glands is limited to the hairless pads of the feet. For the activity of the sweat-glands there is then substituted another physical mechanism, also under the control of the sympathetic, namely, the erection of the hairs of the fur, which also increases the heat-loss.

This difference in the physical mechanism of heat regulation is of import-

ance in the present instance, because one of the characteristic features of true hyperpyrexial heat-stroke in man is the suppression of sweating. How this is brought about is not clear. Hearne (3, 4) attributes it to an exhaustion of the sweat-glands. It is difficult to understand why such an exhaustion should occur, since the secretion of sweat does not involve the formation of a specific product of secretion by the gland-cells, but is simply the secretion of water in which about 0·5 per cent. of sodium chloride and a trace of urea are dissolved. But there is evidence that the activity of the sweat-glands is under the influence of inhibiting factors. Pembrey (9) points to the familiar evidence of the suppression of sweating at the onset of many fevers. He suggests that this is due to a paralysis of the secretory mechanism of the glands produced by the circulating toxins, and that in heat-stroke some unusual product of metabolism acts in a similar way. Several authors have claimed to have demonstrated the existence of inhibitory nerve-fibres to the sweat-glands (11). Of most interest in this connection is an old observation of Luchsinger's (6)—that the external application of heat may inhibit the activity of the sweat-glands. Thus, if on a warm day one hand be held for ten minutes in water at 45° to 50° C., while the other is immersed in water at 15° to 30° C., and exercise is then taken, the hand which was in water at the lower temperature commences to sweat at once, the other not for some considerable time. Experiments on the effect of T.H.N., under different conditions of the temperature of the environment, on animals capable of regulating their heat-loss by sweating might give further information on this point.

Whichever explanation may be found to be correct, the suppression of sweating in heat-stroke is of importance because it is in itself sufficient to bring about a rise of body-temperature, as the observations of Tendlau (16) showed. These were made on an individual who suffered from a congenital deficiency of sweat-glands, and whose temperature on doing heavy muscular work or on merely sitting in the sun would rise to 40° and 41° C. But the condition of hyperpyrexial heat-stroke comprises more than a rise in the body-temperature. There is an early stage which points to a general stimulation, and the symptoms of which are very similar to those of sympathetic stimulation produced in man by small doses of T.H.N. So far as one can judge from the clinical data available, this condition occurs at ranges of the body temperature which do not produce a similar effect in normal men whose temperature has been artificially raised by being exposed to a moist warm atmosphere. The difference is the same as that existing between a rabbit suffering from T.H.N. fever and a rabbit the body-temperature of which has been raised by over-heating. As Stern (13) points out, the latter lies stretched out in the cage with dilated cutaneous vessels and relaxed muscles, avoiding every movement. The former is restless, excited, "stamps," and its cutaneous vessels are constricted. The rise in body-temperature is not therefore in itself an adequate explanation of all the features of hyperpyrexial heat-stroke,

and from this point of view, too, it is necessary to assume the existence of an intrinsic factor.

The conception of hyperpyrexial heat-stroke presented in this paper is, then, that it is due to the combination of an extrinsic factor, namely the deficient cooling due to the conditions of the environment, and an intrinsic factor, namely the hyperactivity of the sympathetic system resulting from the over-activity of the thyroid-adrenal apparatus. This conception recalls the explanation of hyperpyrexial heat-stroke given by van Gieson, Ewing and others (9), who postulated the existence of an autogenetic poison. The question now remains how this overactivity of the thyroid-adrenal apparatus is elicited. An explanation may be found from a consideration of the limitations of the thyroid adrenal apparatus as a heat-regulating mechanism. It is very efficient as long as the temperature of the body has to be kept on a level considerably higher than that of the environment. For not only can its activity increase the heat-production, it can also, if sufficiently great, diminish the heat-loss by the constriction of the cutaneous blood-vessels. This inhibits the activity of the sweat-glands, which might otherwise occur and increase the heat-loss. But as the rate of cooling diminishes with a rise of the temperature of the environment, or with a change in the other factors which determine it, as Leonard Hill (5) has shown, the thyroid-adrenal apparatus has to contend with an increasingly difficult problem. It cannot by its activity diminish the heat-production, and it could only increase the heat-loss if it could stimulate the sweat-glands alone, without at the same time producing the other effects of sympathetic stimulation. Under these circumstances the slightest activity of the sympathetic system would lead to fatal results. There must exist, therefore, a mechanism which is capable of restraining the activity of the thyroid-adrenal apparatus, and the relaxing effect of a hot climate, and especially a hot moist climate, may be looked upon as the psychological expression of such a damping mechanism. Whether this restraint is produced by an actual inhibition of the secretion of adrenin, or by an action on the sympathetic nerve-endings which renders them less sensitive, or in yet another way, is not known. It may be pointed out in passing that there are other facts which demand the assumption of a restraining mechanism as an explanation. Since the adrenal medulla secretes adrenin as the result of sympathetic stimulation, it should do so also as the result of the presence in the blood of adrenin. If that were so the secretion of adrenin by the gland, when once initiated, should lead to a continued secretion of adrenin which should go on until the load of the gland is exhausted. We know, however, that this does not occur—at any rate in the normal animal. In hyperpyrexial heat-stroke this restraining mechanism breaks down and such a vicious circle is initiated. This conclusion is in agreement with the results obtained by Sutton (14) in his experiments made on himself on the effect of a hot moist atmosphere on the gaseous metabolism and the temperature of the normal

human organism, and affords an explanation of the effects observed by him. Sutton states that, "once the balance of the mechanism of heat-regulation has been definitely upset by high external temperature combined with almost total abolition of heat-loss in evaporation a vicious circle is established. The internal temperature rises, and as a result the oxidation processes and therefore the production of heat also increases, so that the body-temperature rises still further, and so on. Once the ball has been set rolling nothing seems to check its progress, and it slowly but surely gathers speed." Very significant, from the point of view developed in this paper, is the rise of the respiratory quotient, observed by Sutton, from 0·77 at the beginning of the experiment to 0·916 at the end, which indicates according to his calculation an increase of carbohydrate consumption to at least five to six times its previous amount. From what we now know of the relation of the activity of the thyroid and adrenal glands to carbohydrate metabolism, which has been briefly summarised at the outset of this paper, this increased oxidation of carbohydrates indicates a hyperactivity of these glands. The vicious circle referred to by Sutton is this hyperactivity of the thyroid-adrenal apparatus which we postulated as the intrinsic factor in hyperpyrexial heat-stroke.

The view put forward on the nature of heat-stroke has an obvious bearing on the treatment of the condition. The paramount importance of reducing the body-temperature by drastic cooling is obvious and generally recognised. But this treatment is, as a rule, instituted only when the condition of heat-stroke has fully declared itself, and the temperature has risen considerably. In that case this treatment only combats the symptoms and is accordingly not very successful. But, as Hearne points out (3, 4), hyperpyrexial heat-stroke gives ample warning of its onset (according to him from one to forty-eight hours), and he contends that the onset of heat-stroke can with certainty be completely aborted if treatment is instituted in this prodromal stage. Rogers also emphasises the importance of an early and prophylactic treatment. The views put forward in this paper support Hearne's contention and so do the experiments. We refer here particularly to the experiments in which mice, after a given dose of T.H.N., developed experimental heat-stroke when kept in a crowded cage, but showed no ill-effects, and not even a rise in temperature, if kept under conditions admitting of efficient cooling.

In addition to reducing the temperature, there are accessory methods of treatment which may be discussed.

The injection of saline or other fluids has been recommended. That, however, is clearly contra-indicated in this condition. Venesection, which has also been recommended, is not open to the same objection, but it is not clear how it can combat the condition beyond relieving the symptoms. Morphine has been recommended as a sedative. This drug acts differently in different species of animals, so that in this case deductions from observations on animals may not necessarily be valid for man. Still it is important to note

that in mice the injection of morphia, together with T.H.N., did not reduce the mortality when compared with animals which had received the same dose of T.H.N. alone under the same conditions. On the contrary, so far as the experimental evidence goes, it shows that after morphine the mortality is higher and the animals die sooner, as the following record shows:

Experiment.	No. of mice.	Dose of morphine in mgrm.	Dose of T.H.N. in mgrm.	Result.
I	3	4 mgrm.	3 mgrm.	After 2½ hrs. all three dead.
	2	—	3 „	After 2½ hrs. both mice alive, but one very ill. Dead after 3 hrs. One mouse survived.
II	3	0·5 mgrm.	2·5 „	After 2 hrs. all three mice dead.
	3	—	2·5 „	After 2 hrs. one dead, two ill. Two mice survived.
III	4	3 mgrm.	2·5 „	After 2 hrs. three mice dead. The fourth mouse died half-an-hour later.
	4	—	2·5 „	After 2 hrs. one mouse died; two others died within the next half hour. One mouse survived.
	4	3 mgrm.	—	All four mice alive and well throughout.

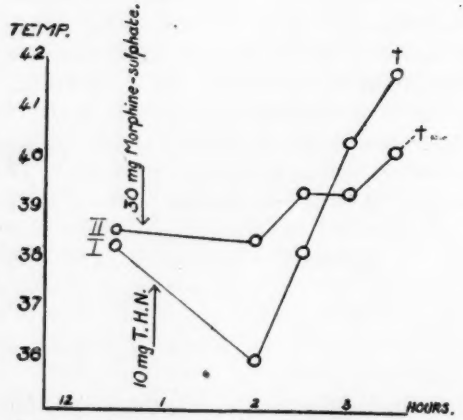

FIG. 1.—Effect of morphine on T.H.N. fever in rats. Curve 1: Temperature of control animal. Curve 2: Temperature of morphinised animal. Both received 10 mgrm. T.H.N. at the same time. † indicates death of the animal.

Morphine modifies the action of T.H.N. so that the temperature rises without being accompanied by an excitement of the animal. The symptoms are thus less violent, and the appearance of a beneficial effect may be simulated in man and may have led to its use. But from the experimental evidence its use is strongly contra-indicated. The effect of morphine in the temperature curve of T.H.N. hyperpyrexia is given in Fig. 1.

For the treatment of experimental T.H.N. heat-stroke chloral is a much more suitable drug. In mice and rats I have found it possible to delay the onset of experimental T.H.N. heat-stroke by doses of chloral which will produce

a slight anæsthesia if given alone, and even to abort it completely. The

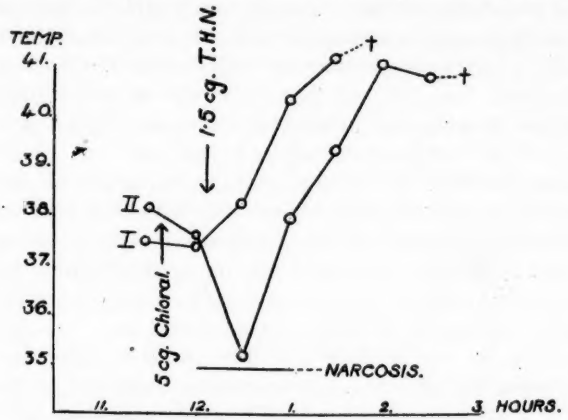

FIG. 2.—Effect of chloral on T.H.N. fever in rats. Curve 1: Temperature of control rat. Curve 2: Temperature of chloralised rat. Both animals received 15 mgrm. T.H.N. at the same time. The period during which chloral exercised its narcotic effect is indicated by the horizontal line marked "narcosis."

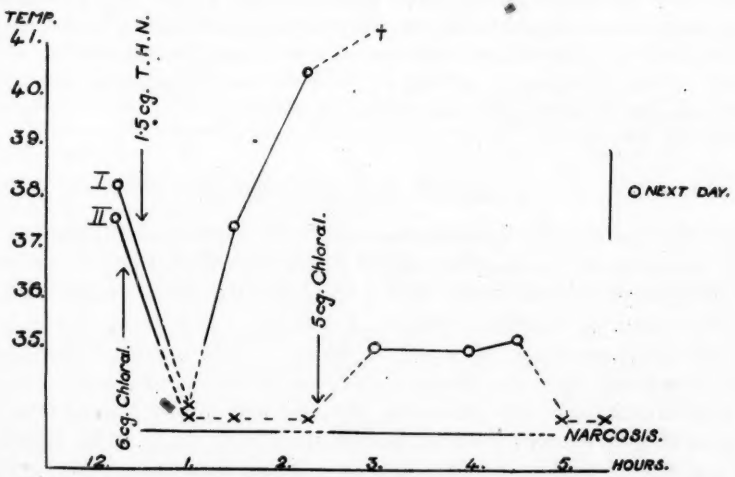

FIG. 3.—Prolonged effect of two doses of chloral on T.H.N. fever in rats. Curve 1: Temperature of control rat. Curve 2: Temperature of chloralised rat. The thermometer did not register temperatures below 35°C. Such observations are indicated by the sign ×.

results of three such experiments on rats are given in Figs. 2 and 3. There appears to be a physiological antagonism between chloral and T.H.N.

So long as the narcotic effect of chloral predominates the animal is asleep and shows none of the effects of T.H.N. which are visible in the non-chloralised animal; the temperature remains sub-normal, even after the injection of a dose of T.H.N., which proves fatal to a normal animal. On the other hand, the effect of chloral does not last as long in an animal which has received T.H.N. as it does in a normal animal, and as soon as the narcotic effect of chloral passes off the temperature begins to rise, and the animal may still die in hyperpyrexia (see Fig. 2). But if the narcotic effect of chloral is maintained by means of a second dose, the experimental heat-stroke may be completely aborted (Fig. 3). In the experiment represented by Fig. 3 the prolonged effect of chloral prevented the temperature from rising, and the animal was alive and well for three months after the experiment.

The effect of chloral is probably not a specific one, but due to the condition of anæsthesia produced by it. For there is experimental evidence that in anæsthesia the effect of a given dose of adrenin is altered and less forcible than in a normal animal. And Fawcett and Hale White (2) found that rabbits in ether anæsthesia not only failed to respond to the injection of T.H.N. with a rise of temperature, but that this drug was not even able to prevent or reduce the fall of temperature produced by the anæsthetic. The significant difference between the action of morphine and chloral lies in the fact that morphine, while exercising its characteristic effect in subduing the excitement of the animal, fails to prevent the rise in body-temperature and the death of the animal, and perhaps even accelerates the lethal issue. Chloral, on the other hand, as long as its effect lasts, suppresses not only the visible symptoms of T.H.N., but also the fever.

SUMMARY AND CONCLUSIONS.

By the injection of suitable doses of β-tetrahydronaphthylamine a prolonged sympathetic stimulation associated with an over-activity of the thyroid-adrenal apparatus is produced. The increased heat-production and diminished heat-loss resulting therefrom induces a pyrexia, which from its origin it is proposed to designate as "sympathetic fever." The height of the pyrexia is chiefly dependent upon the climatic conditions of the environment. Exposure to a cool environment, by increasing the heat-loss, diminishes the height of the fever or may prevent the body-temperature from rising. An interference with the rate of cooling by exposure to a warm atmosphere or by keeping animals crowded together elicits a rapidly fatal hyperpyrexia. This hyperpyrexia, from the post-mortem findings and the conditions under which it occurs, presents such a striking resemblance to hyperpyrexial heat-stroke as it occurs in man that it may be described as an "experimental heat-stroke." This resemblance is confirmed by a comparison of the premonitory symptoms of hyperpyrexial heat-stroke in man and the effect in man of very small doses

of β-tetrahydronaphthylamine. The explanation of hyperpyrexial heat-stroke in man is, therefore, that an extrinsic factor, namely, deficient cooling due to the conditions of the environment, acts upon an organism which is under the influence of prolonged sympathetic stimulation due to an excessive activity of the thyroid-adrenal apparatus.

The bearing of this view on the treatment of heat-stroke is discussed.

It gives me great pleasure to acknowledge my indebtedness to Dr. K. G. Hearne for much valuable information on hyperpyrexial heat-stroke in man derived from his extensive experience of this condition as it occurred in Mesopotamia among the troops.

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THE SEROLOGICAL CLASSIFICATION OF MENINGOCOCCI.

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SEROLOGICAL differences among meningococci were first clearly established by Dopter (1). He showed that cocci might be isolated from the cerebro-spinal fluid which did not agglutinate with meningococcus serum, although they agglutinated with a serum made from themselves. He called these strains "parameningococci," and later subdivided these into further serological sub-groups.

Arkwright (2) also, in this country, found serological differences among meningococci, and was able to divide his strains into two main groups, which, however, were not distinct, but merged one into the other.

At the beginning of the war Gordon and Murray (3) examined a large number of cultures of meningococci which were obtained from cases of "epidemic" cerebro-spinal fever in the Army. By preparing agglutinating sera from single strains they found that four different monovalent sera were sufficient to agglutinate all specimens, and on this basis divided them into four "types."

In numerous later papers Gordon (4) has held that his four types are serologically distinct, as shown by the sharp result of absorption tests, and that practically all strains from "epidemic" cases could be relegated to one of his "types."

The "distinctness" of the serological types was disputed by Griffith and Scott (5), working under the late Local Government Board upon civil cases. By using monovalent sera they were only able to distinguish two main groups, which, however, overlapped, and they held that a serological classification such as Gordon's depended for its sharpness upon an arbitrary selection of standards for the different "types."

The attitude of Gordon was largely supported by Fildes and Baker (6), who, working upon the "epidemic" of 1916–17 in the Navy, agreed that all (except one) strains of meningococci obtained from the cerebro-spinal fluid were capable of being referred to one or other of Gordon's four types. With regard to the criticisms of Griffith and Scott, they admitted that the serological relationship between types I and III on the one hand and types II and IV on the other were such that confusion between them might arise from the selection of arbitrary standards, but held that the group I–III differed so markedly from the group II–IV that no arbitrary selection of standards would lead to confusion between them. They considered that two main serological groups of meningococci existed, and that each group contained two outstanding serological characters which might be combined together in one bacterium, and thus produce "intermediate" serological varieties.

Gordon himself (4) admitted that the sporadic civil cases of Griffith and Scott might show a less sharp serological differentiation than "epidemic" cases, but denied that in these latter there was any relationship between the types so close that they were capable of being confused by an arbitrary selection of standards.

A study of a large number of epidemic military strains was made by Tulloch (6), and he was able to place 101 out of 107 strains into one or other of Gordon's types. He found, however, that type II cases could not always be so identified upon investigation with one standard only, but he required to select three strains of standards before this could be achieved.

It appeared, therefore, that even among military strains there were some which could not be identified by Gordon's methods, while type II at least could not be looked upon as a uniform and definite variety.

The investigation of cases in the Navy was continued during the period 1917–18. The number of cases during that winter was smaller than in the previous winter and thus they might be described as "sporadic," but there was not really any essential difference in the curve of incidence. It was found that the number of cases which could not be classified as Gordon's types was larger, and the present paper represents a study of the value of "type" classification upon the basis of these cases.

It was decided that the whole question of selection of standards must be avoided and that the absorptive power of one strain must be tested with a serum which "offered" agglutinins of all shades of type, instead of with a so-called "monotypical" serum, which was not "monotypical" at all. Such a serum, capable of agglutinating all types of meningococci, when absorbed with strain A would lose all agglutinins for strain A, and if any other strains (B, C, D) were similarly affected they might be classed in the same group; but it must be shown that absorption with B, C or D did not upset this grouping before its correctness was accepted.

The serum which was selected was a horse-serum prepared for treatment

purposes, and this has a particular value for the present experiment, because the agglutinin response of the horse to an injection of a meningococcus is very broad and polyvalent. In a young rabbit the first agglutinins to appear are more "specific" to a particular strain, while in the horse the repeated inoculation of one strain will produce agglutinins for all types of meningococci. This is well shown in a test of the monovalent meningococcus serum prepared for the Medical Research Committee at Cambridge. Tested in June, 1918, each "type" serum agglutinated all "type" cocci up to a dilution of 1 in 400 to 800.

The selection of a horse-serum, therefore, ensured that agglutinins for all strains of meningococci would be present, which was necessary for the success of the experiment.

A collection of meningococci from the cerebro-spinal fluid was made and each of these strains was used to absorb the polyvalent anti-meningococcus serum. The serum was shown to exert an agglutinating effect upon all, and this action was again tested after the agglutinins for each strain separately had been removed. In this matter it was shown that absorption with one strain removed the agglutinins for itself and for certain others, and such could then be grouped together as being of similar serological character. Such grouping did not depend in any way upon arbitrary selection of standards.

Twenty-one different strains of meningococci were tested. Each was grown upon a hæmoglobin-serum-agar medium, and thick suspensions were made of each. These suspensions were diluted with carbolic saline to an opacity corresponding to 8000 million cocci per c.c. and kept as stock.

The polyvalent serum was obtained from the Rockefeller Institute and was dated July 25th, 1917, the experiment being carried out in January, 1918. The serum was diluted in bulk to 1 in 50 and the same serum and suspensions were used throughout the whole experiment.

Equal parts of the diluted serum and stock suspension were mixed together and kept in the ice chest for eight hours and then in the incubator for eight hours. The tubes were thoroughly centrifuged and the supernatant fluid taken off. This serum, now diluted 1 in 100, was then used to agglutinate all strains of cocci, the stock suspensions being diluted 1 in 4 for this purpose. The ultimate dilutions of serum were 1 in 200, 1 in 400, 1 in 800, and 1 in 1600. The unabsorbed serum was also put up with the same suspensions of cocci in the same dilutions, and the results with the absorbed serum were compared with those of the unabsorbed.

The following is an example of such an experiment:

Flexner Serum absorbed with Strain St., Ds., and Hr. separately and tested upon 21 strains.

Strains on which the absorbed serum was tested.	Serum absorbed with strain. St. 1 in				Ds. 1 in				Hr. 1 in				Not absorbed. 1 in			
	200	400	800	1600	200	400	800	1600	200	400	800	1600	200	400	800	1600
St.	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	1
Ds.	0	0	0	0	0	0	0	0	2	2	2	1	2	2	2	2
Hg.	1	0	0	0	0	0	0	0	2	2	2	1	2	2	2	2
Re.	1	0	0	0	2	1	0	0	2	2	2	0	2	2	2	1
An.	0	0	0	0	0	0	0	0	2	2	2	1	2	2	2	1
Hr.	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0
Js.	0	0	0	0	0	0	0	0	2	2	1	0	2	2	1	0
Le.	0	0	0	0	0	0	0	0	2	0	0	0	1	1	0	0
Tr.	2	2	2	2	2	0	0	0	2	2	2	2	2	2	2	2
Ck.	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	1
Ws.	2	1	1	0	2	1	0	0	2	0	0	0	2	2	0	0
Cr.	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	2
Hn.	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	1
Fr.	2	2	2	0	2	2	2	0	2	2	2	0	2	2	2	0
Kl.	2	2	2	0	2	2	2	1	2	2	2	1	2	2	2	1
Ce.	2	1	0	0	1	1	0	0	2	1	0	0	2	1	0	0
Ct.	2	2	2	1	2	2	1	1	2	2	2	1	2	2	2	1
Br.	2	2	2	1	2	2	2	2	2	2	2	0	2	2	2	1
Hs.	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2
P.	2	2	2	0	2	2	2	0	2	2	1	0	2	2	2	0
Bn.	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0

2 = complete agglutination with the dilution indicated.
1 = incomplete agglutination with the dilution indicated.
0 = no agglutination with the dilution indicated.

From these figures the degree of absorption was calculated in the following way :

When, upon comparison of the agglutinating power of the absorbed serum with that of the unabsorbed, it was found that no removal of agglutinins had taken place or that less than half of the full content of agglutinins had been removed, the result is marked as 0 against the strain whose agglutinins were under question ; thus in the following table no Tr. agglutinins were removed by absorption with St. When half of the agglutinins were removed the sign + is used, and when over half, the sign +.

Strains on which the absorbed serum was tested.	Serum absorbed with strain— St.	Ds.	Hr.
St.	+	+	0
Ds.	+	+	0
Hg.	+	+	0
Re.	+	+	0
An.	+	+	0
Hr.	+	+	+
Js.	+	+	0
Le.	?	?	?
Tr.	0	+	0
Ck.	0	0	0
Ws.	0	0	+
Cr.	0	0	0
Hn.	0	0	0
Fr.	0	0	0
Kl.	0	0	0
Ce.	0	0	0
Ct.	0	0	0
Br.	0	0	0
Hs.	0	0	0
P	0	0	0
Bn.	+	+	+

Note.—The agglutinability of the particular suspension of strain Le. used was so slight that no inferences could be drawn.

From this table it may be deduced provisionally that Hr. in the serological sense is similar to Bn., because the agglutinins for Bn. are removed by absorption with Hr.; and that St. is not identical to Ds., because absorption with Ds. removes agglutinins for Tr., while absorption with St. does not. The full significance of the grouping cannot, however, be apparent until a larger number of strains have been tested.

In the next table the whole twenty-one strains are shown, and the extent of the absorption from Flexner serum noted in each case. These figures were arrived at by carrying out two or three absorption experiments with the unabsorbed control daily until the series was complete. The same stock materials were used throughout.

The cases are rearranged in order to show the serological groups into which they fall. Strains which absorb similar or almost similar agglutinins are grouped together.

Cocci Used for Absorbing Flexner Serum.

Cocci upon which the Absorbed Serum was Tested.

	Tr.	Hg.	Ds.	An.	Js.	Le.	St.	Re.	Hr.	Bn.	Kl.	Ce.	Ws.	Cr.	Ct.	Ck.	Hn.	Hs.	Fr.	P.	Br.
Tr.	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hg.	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ds.	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
An.	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Js.	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Le.	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
St.	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Re.	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Hr.	+	+	+	+	+	0	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0
Bn.	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	0	0	0	0	0	0
Kl.	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0
Ce.	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0
Ws.	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	+	+	0	0	0	0
Cr.	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	0	0	0
Ct.	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	0	0	0
Ck.	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	0	0	0	0
Hn.	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	0	0
Hs.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	+	+	0
Fr.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	0
P.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	+	+	0
Br.	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	+	0	+	0	+	+

It will be observed that most of the strains are capable of being arranged into four groups, which are shown enclosed in squares. They may be referred to as groups A, B, C, D, from above downwards. In the main, the absorption by one strain is confined to the agglutinins for the other members of the same group. There is very little absorption in the portions of the table outside the groups, except where groups A and B abut, and where groups C and D abut. Group A, consisting of eight members, is connected with the small group B by the intermediate group containing strains Hr. and Bn. These two bacteria absorb agglutinins for themselves only, but both the large group A and the small group B absorb the agglutinins for Hr. and Bn. in addition to their own agglutinins. Thus these two bacteria must be looked upon as intermediate forms between the more distinct members of groups A and B. In fact, groups A and B and the intermediate forms may be looked upon as one " super-group."

Similarly group C, containing five members, is related to group D containing four, as shown by the slight degrees of absorption in the neighbourhood of their junction.

From this experiment, therefore, it appears justifiable to assume that

there are two practically distinct serological groups among meningococci, and that each group consists of bacteria with two main serological characters, and also bacteria with these characters merged into one another to a greater or less extent.

In order to determine the relation of these groups to Gordon's types, cultures of his standard cocci, with which he had supplied me, were introduced into the experiment.

The strain Le. was Gordon's type I standard. Ce. was his type III standard. Hn. was his type II standard, although later he supplied me with Fr. as type II. Fr., however, in our classification falls into another group (type IV). Hs. and P. were both his type IV strains. With regard to Ct., this strain was used by me as a type IV standard on the strength of its reaction with type IV serum supplied to me by Col. Gordon, but it is now shown to be in a different group to Gordon's type IV strains and in the same group as his type II strains.

Thus it may be said that Gordon's types I and III correspond to the main characters of super-group I, but they do not account for the intermediate strains Hr. and Bn. Similarly his types II and IV correspond to the main features of super-group 2, but his classification does not suggest the marked degree of relationship between his two types. In fact, there is evidence of some confusion of standards owing to the closeness of relationship, which leads to type IV cocci being called type II, and type II cocci being called type IV.

Thus it will be seen that the grouping into Gordon's four types does not depend upon a selection of standards, since it emerges from this experiment in which no standards exist. But at the same time the relationship between types II and IV is so close that the change of a standard culture may even lead to a change in the "type" of the organism, and in this sense the selection of standards for sub-group characters is arbitrary and may lead to confusion. Further, even among epidemic cocci (Naval), strains may occur commonly which do not belong distinctly to any of Gordon's types, but fall between them.

The existence of intermediate strains is important because they correspond closely with cocci which are often isolated from the naso-pharynx and discarded as not meningococci because they do not agglutinate with "type" serum nor absorb "type" agglutinins. The "intermediate" cocci behave in the same way as shown in the following tables:

The Effect of "Type" Serum upon "Intermediate" Cocci.

Suspension of strains.		Agglutinating serum. Type I (Hg.). 1 in				Type III (Ce.). 1 in			
		100	200	400	800	100	200	400	800
An.	Type I	2	2	2	2	2	1	0	0
Re.	,,	2	2	2	2	2	1	0	0
Js.	,,	2	2	2	0	0	0	0	0
Hg.	,,	2	2	2	2	1	1	0	0
Cl.	,,	2	2	1	0	0	0	0	0
Le.	,,	2	2	2	1	1	0	0	0
Ds.	,,	2	2	2	2	2	1	0	0
St.	,,	2	2	2	2	2	1	0	0
Cn.	Type I–III	1	0	0	0	1	0	0	0
Bn.	,,	1	0	0	0	1	0	0	0
Hr.	,,	1	0	0	0	1	0	0	0
Kl.	Type III	2	1	0	0	2	2	2	1
Ce.	,,	0	0	0	0	2	1	0	0

In this experiment it is seen that type I and III sera have usually an agglutinating action on both types, but each agglutinates its own type to a higher dilution. The three intermediate cocci are hardly agglutinated by either serum.

The Effect of Type Serum after Absorption with "Intermediate" Cocci.

					1 in			
					100	200	400	800
Type I serum (Hg.) absorbed with	Cn.	and tested on	type I	(Le.)	2	2	2	0
,, ,, ,,	Bn.	,, ,,			2	2	2	0
,, ,, ,,	Hr.	,, ,,			2	2	2	0
,, ,, ,,	An. (type I)	,, ,,			1	0	0	0
,, ,, ,,	Ce. (type III)	,, ,,			2	2	2	0
,, ,, unabsorbed		,, ,,			2	2	2	0
Type III serum (Ce.) absorbed with	Cn.	and tested on	type III	(Kl.)	2	2	2	0
,, ,, ,,	Bn.	,, ,,			2	2	2	0
,, ,, ,,	Hr.	,, ,,			2	2	2	0
,, ,, ,,	An. (type I)	,, ,,			2	2	2	0
,, ,, ,,	Ce. (type III)	,, ,,			0	0	0	0
,, ,, unabsorbed		,, ,,			2	2	2	0

Thus the intermediate cocci neither remove type agglutinins from the sera nor are they obviously agglutinated by the type sera. They are, however, like the naso-pharyngeal cocci agglutinated by Flexner serum.

From these results it is clear that these intermediate cocci, if found in the throat, would be classed as "non-epidemic" if the type sera are relied upon for diagnosis, and this is one of the great technical fallacies in trying to rid a population of "carriers."

The term "non-epidemic" has become practically synonymous with "non-pathogenic." This was due to the fact that Gordon (and Fildes and Baker) found very few cocci in the cerebro-spinal fluid when the epidemic was more wide-spread, which did not conform to Gordon's types. Thus it was held that cocci from the naso-pharynx which did not conform to Gordon's types might be overlooked as harmless.

This was probably quite a justifiable assumption to make under the then prevailing circumstances, but it clearly has little scientific foundation, and if agglutination by four mono-typical sera is taken as the basis for the diagnosis of meningococci for the future, serious error (in the bacteriological sense) is liable to be introduced.

CONCLUSIONS.

(1) Meningococci may be divided into two distinct serological groups. Each group is capable of being analysed into two main serological sub-groups, and all cocci in the main group display each of these sub-characters to a greater or less extent.

(2) This classification is not dependent upon an arbitrary selection of standards.

(3) A selection of standards for closely-defining sub-group characters is an arbitrary proceeding, and may lead to confusion if one and the same strain is not maintained as standard.

(4) The use of monovalent sera manufactured from arbitrary standards of sub-group characters will fail to reveal certain cocci as meningococci, although these may have been isolated from the cerebro-spinal fluid of a case of the disease.

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ON THE TESTING OF RENAL EFFICIENCY, WITH OBSERVATIONS ON THE "UREA COEFFICIENT."

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UNTIL recently the condition of the kidneys in patients suffering from renal disease was estimated chiefly on the results of an examination of the urine for protein and casts. While it was recognised that albuminuria might sometimes be present in otherwise healthy patients, the symptom was, on the whole, generally considered to be of great significance, indicating in the majority of cases a more or less grave lesion of the kidneys. When the albuminuria was accompanied by casts, clinical observers in general accepted the view that definite renal disease was present. Recent work, however, has shown that albuminuria is much more often met with than has hitherto been recognised, and it appears certain that many of our views concerning the importance of this condition must be subjected to a radical change.

In civil life it is difficult to ascertain the incidence of albuminuria in the population, but that it must be very much greater than has hitherto been supposed is evident from the results obtained in persons undergoing military service. In 50,000 apparently fit soldiers who had just completed their training, MacLean (1) found protein present in the urine of nearly 6 per cent. While many of these men showed only a small amount of protein, well-marked albuminuria was present in over 2 per cent., while in nearly 2 per cent. casts of various kinds were present as well. When it is considered that all these men had passed a medical examination before enlisting and that they represented the younger part of the population, it appears certain that an investigation on a similar scale of the average civil population would show an even greater incidence. All these men had just undergone several months of comparatively severe military training, yet they had not broken down or showed in any way that the presence of albuminuria rendered them less efficient than other men. That this large incidence of albuminuria was not connected with training was proved by an examination of 10,000 newly joined

recruits. Here the incidence was, if anything, rather larger. This and other considerations compel us to modify our views on albuminuria; the condition may, of course, be an indication of a grave renal lesion, but albuminuria *per se* is not necessarily of any great importance, and is often not incompatible with the enjoyment of perfect health even in certain cases where it is known to have resulted from a previous attack of nephritis.

Further, as an index of renal efficiency the presence of albuminuria, even when associated with casts, is of little value in helping us to arrive at a conclusion in any given case. Generally speaking, the presence of very large amounts of protein together with granular and epithelial casts in the urine is, of course, an indication that the kidneys are more or less seriously involved, but even in such cases it is impossible to gauge to what extent these organs are still capable of performing their normal functions. This, after all, is the important point to decide, but this knowledge is not to be obtained by an examination of the urine for protein and casts. Other indications are, of course, available at the later stages of chronic renal disease, such as involvement of the cardio-vascular system, the low concentration of urea in the urine, the presence of œdema, etc., but in many cases of moderate severity no help can be obtained in this direction. In very severe cases the clinical condition alone is unfortunately often so grave as to give all the information required. On the other hand, it is not an uncommon occurrence in hospitals to have patients admitted who had been only very slightly indisposed before admission, but who rapidly become uræmic and die in convulsions a few days or a few weeks later. In such cases the clinical condition may remain good until a short time before the fatal issue. Usually, however, the gravity of the condition can be ascertained by an examination of the blood for non-protein nitrogen or urea. In severe cases the blood urea is found to be far in excess of the normal 20 to 40 mgrm. per 100 c.c. of blood. In acute cases also the estimation of blood-urea supplies the necessary information as to the progress of the patient. Unfortunately, however, the examination of the blood gives little or no help in the large number of cases in which the condition has not yet developed to an extreme degree, but in which the kidneys, though efficient enough to prevent an accumulation of urea in the blood, may yet be gravely involved. Such cases are very commonly met with at the present time in men who contracted war nephritis in the army; the presence of protein and casts in the urine does not help us, and in the great majority of such cases experience shows that the blood urea is normal.

THE NEW UREA CONCENTRATION TEST.

In endeavouring to ascertain the efficiency of the kidneys in such patients we have made use of the various dyes and other tests used so extensively in America, but on the whole, for one reason or another, we have not found them

very satisfactory. We found, however, that when a large dose of urea was administered to a patient with defective kidneys the patient was *incapable of excreting urine with a high urea concentration.* Further, the degree of concentration appeared to be in direct relationship to the involvement of the kidneys. Reference has already been made to this test (2), but since that time we have been able to apply it in over 1200 persons suffering from the effects of war nephritis. The results have been most satisfactory, and the test has proved itself to be of very great practical value in assessing military patients for pensions. It is at present being extensively employed by the Ministry of Pensions for this purpose. The details of this test are as follows:

(1) The patient empties his bladder and immediately afterwards takes by mouth 15 grm. of urea dissolved in about 100 c.c. of water. The solution may be flavoured with a few drops of tincture of orange.

(2) One hour after taking the urea he passes water. This sample is kept for estimation of urea percentage.

(3) Two hours after taking the urea he again passes water. This sample is also kept for estimation of urea percentage.

The test is exceedingly easily carried out, the urea being estimated by the ordinary hypobromite solution. If the percentage of urea exceeds 2, the kidneys may be taken as fairly efficient; if below 2, the condition is unsatisfactory, and the lower the concentration the more serious the lesion. Cases with a concentration lower than 1 per cent. are rare, but many moderately severe cases are unable to concentrate to more than about 1·4 to 1·5 per cent.

The urea should be estimated by some form of apparatus which gives the volume of gas evolved. The ordinary apparatus giving percentage of urea directly is, in our experience, as a rule, not very accurately graduated. We use an ordinary 50 c.c. burette to act as a nitrometer, and take 4 c.c. of urine and 25 c.c. of hypobromite solution made up in the ordinary way. The open end of the burette is connected with a T-piece which communicates above with a glass tap or piece of rubber tubing fitted with a clip. By means of the side arm it is connected by rubber tubing with the bottle containing the urine and hypobromite solution. The lower end of the burette is attached by rubber tubing to a small bell-shaped vessel to hold the water displaced by the evolved nitrogen. By adjusting the level of the water in the nitrometer before and after an experiment in the ordinary way, satisfactory results can be obtained with the minimum of trouble.

The percentage of urea equivalent to the amount of gas evolved may be ascertained directly from the following table. This table was compiled by taking several specimens of urine and estimating the urea present by the very accurate urease method. Samples of the same urines were then analysed by the hypobromite method, and the amounts of nitrogen evolved compared with the amounts of urea known to be present in the particular samples. The results were then compared and an average taken.

Table showing the Percentage of Urea equivalent to c.c. of Nitrogen evolved.

(4 c.c. urine + 25 c.c. hypobromite solution used.)

Nitrogen c.c.	Urea per cent.	Nitrogen c.c.	Urea per cent.	Nitrogen c.c.	Urea per cent.	Nitrogen c.c.	Urea per cent.
8·0	0·5	22·4	1·40	36·8	2·30	51·2	3·20
8·8	0·55	23·2	1·45	37·6	2·35	52·0	3·25
9·6	0·60	24·0	1·50	38·4	2·40	52·8	3·30
10·4	0·65	24·8	1·55	39·2	2·45	53·6	3·35
11·2	0·72	25·6	1·60	40·0	2·50	54·4	3·40
12·0	0·75	26·4	1·65	40·8	2·55	55·2	3·45
12·8	0·80	27·2	1·70	41·6	2·60	56·0	3·50
13·6	0·85	28·0	1·75	42·4	2·65	56·8	3·55
14·4	0·90	28·8	1·80	43·2	2·70	57·6	3·60
15·2	0·95	29·6	1·85	44·0	2·75	58·4	3·65
16·0	1·00	30·4	1·90	44·8	2·80	59·2	3·70
16·8	1·05	31·2	1·95	45·6	2·85	60·0	3·75
17·6	1·10	32·0	2·00	46·4	2·90	60·8	3·80
18·4	1·15	32·8	2·05	47·2	2·95	61·6	3·85
19·2	1·20	33·6	2·10	48·0	3·00	62·4	3·90
20·0	1·25	34·4	2·15	48·8	3·05	63·2	3·95
20·8	1·30	35·2	2·20	49·6	3·10	64·0	4·00
21·6	1·35	36·0	2·25	50·4	3·15		

It occasionally happens that urea causes a marked diuresis in certain patients who have been imbibing large amounts of fluid immediately before taking the dose. The intake of excessive fluid may of course be prevented in hospital patients, but in the case of out-patients and others who are seen only for a short time it is sometimes impossible to avoid this complication. In such cases the very large amount of urine passed (often 400 to 600 c.c. in two hours) lowers the urea concentration even in kidneys that are capable of concentrating. Here the sample of urine for analysis should not be taken until the end of the third hour after the dose of urea is given. If it is not convenient to detain the patient beyond two hours, or if, as may very occasionally happen, the amount of urine passed in the third hour is also large, the condition may be put down to diuresis, especially in the absence of other symptoms of renal disease. The amount of urine passed during the second hour should not exceed 150 c.c. It is important to note that the passage of very considerable amounts of urine does not necessarily mean a low concentration of urea in the urine, for a quite healthy kidney generally succeeds in concentrating to 2 per cent. or over even after large amounts of fluid have been taken immediately beforehand. The mental condition also seems to play a part sometimes, as in a few cases we have noticed that very nervous patients tend to have a diuresis.

On the whole even in hospital out-patients there is very little trouble in the use of the test. The urea solution, though not pleasant to the taste, never appears to give rise to any untoward symptoms. In testing nearly 1500 cases only one patient rejected the urea by vomiting. The test is a simple one and very easily applied; it requires no special apparatus or facilities, and can be easily carried out in the hospital wards or in general practice.

ROUTINE TESTING OF RENAL CASES.

In testing for renal efficiency it is of course necessary to examine the cardio-vascular system as well as the kidneys. As a routine method we now make use of the following tests in the examination of very large numbers of cases which we are at present carrying out for the Ministry of Pensions. They are all easily applied, do not entail undue detention of the men, and appear to give all the information necessary for a just assessment of the patient's condition. In conjunction with the urea test we use the well-known diastatic test, but in many cases experience shows that this is really unnecessary. It is important to note that the diastatic activity of the urine is of much greater value when obtained from a sample of the twenty-four hours' output than in isolated samples passed at different times of the day. Of all these tests the new urea concentration test is the one which furnishes the most information, and our experience in nearly 1500 cases has led us to attach a very great deal of importance to it. Like every other test its results may in a very small percentage of cases be difficult to interpret, but its general reliability and ease of application should render it of great assistance in clinical medicine and in many surgical conditions as well.

Scheme for Examination of Renal Condition.

(1) Presence or absence of protein. Is amount very large, medium or small?

(2) Presence of casts or other abnormal elements, such as pus, blood, spermatozoa, etc. Nature and amount of casts present.

(3) Diastatic test (normal for healthy patient taken as 6 to 20 units).

(4) Urea concentration test.

(5) Presence or absence of œdema.

(6) Blood-pressure.

(7) Apex-beat position.

(8) Condition of arteries.

(9) General condition of patient.

This scheme is also used at St. Thomas's Hospital for the examination of both medical and surgical patients. In prostate and other operations on the urinary system it is most important to ascertain beforehand with certainty the condition of the kidneys, and we find that the above tests give us all the

information required. In hospital practice we generally estimate the blood urea as well, especially in cases where the other tests indicate a more or less unsatisfactory renal condition.

The following results were obtained from patients: they illustrate cases where renal efficiency was good, fair, and bad. It is interesting to note that in each case fairly large amounts of protein together with large numbers of casts were present.

	Renal condition good.		Renal condition fair.		Renal condition bad.	
	Case 1.	Case 2.	Case 3.	Case 4.	Case 5.	Case 6.
Protein	Large amount	Large amount	Large amount	Very large amount	Fair amount	Medium amount
Casts	Hyaline and epithelial	Hyaline and granular	Hyaline, granular, and epithelial	Hyaline and epithelial	Hyaline, granular, and epithelial	Many epithelial
Diastatic test	20	10	6	4	5	1
Urea test	2·7	3·5	1·4	1·6	1·2	0·85
Œdema	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	Traces
Blood-pressure	126	120	138	128	230	190
Condition of arteries	Good	Good	Good	Good	Slight thickening	Slight thickening
General condition	Fairly good	Good	Fairly good	Fair	Anæmic	Fair (pale)
Blood urea (in mgrm. per 100 c.c. blood)	—	—	23	38	80	144

THE UREA COEFFICIENT.

In estimating the functional efficiency of the kidneys extensive use has been made in recent years, especially in America, of certain formulæ based on the relationship of the concentration of urea in the blood to the concentration and amount of urea in the urine. Widal and Javal (3), working on nitrogenous equilibrium in nephritis, showed that a rise in the blood-urea concentration could be produced by increasing the nitrogen of the diet, that such a rise was followed by a corresponding increase in the elimination of urea, and that ultimately nitrogenous equilibrium was re-established, though the blood-urea remained at a higher level than at the beginning of the experiment. The subject was further investigated by Ambard (4), who, from the

laws which he had formulated for urea excretion, deduced the following constant, K, which he called the coefficient of urea excretion:

$$K = \frac{Ur}{\sqrt{D \times \frac{70}{W} \times \sqrt{\frac{C}{25}}}}$$

When Ur. = Grammes of urea per litre of blood.
D. = Grammes of urea excreted in twenty-four hours.
W. = Weight in kilos.
C. = Grammes of urea per litre of urine.
70 = Standard body-weight in kilos.
25 = Standard concentration of urea in grammes per litre of urine.

Ambard, working with this formula, found the value for K in normal human subjects to be 0·06 to 0·07, or according to F. C. MacLean (5), who worked with the more accurate method of urea estimation, 0·08. MacLean, eliminating the arbitrary standards for weight and concentration in the Ambard formula, employed the simpler expression $\frac{Ur}{\sqrt{\frac{D}{W} \times \sqrt{C}}}$, and obtained in a series of 107 observations of normal subjects figures varying from 0·20 to 0·36. The formula, therefore, gives fairly constant results in normal subjects, and the question arises how far variations from it are a regular feature of the function of the diseased kidney in which urea excretion is impaired.

With impairment of the urea-excreting function of the kidney two possibilities are present. In the first case the excretion by the diseased kidney may be able to keep pace with the formation of urea in the body, and the blood-urea will remain within normal limits even although excretion is not taking place along quite normal lines. In the second case, urea is not excreted as rapidly as it is formed and consequently accumulates in the blood, though equilibrium may be established at a higher level of blood-urea. In such cases blood examination reveals the excess of urea, and it only remains to determine whether the defective elimination is due to a primary kidney defect or whether it is secondary to some other condition, such as cardiac failure. With cases, however, in which there is no retention and in which the blood urea is within normal limits, the blood examination, as already indicated, gives us no information as to the extent of the impairment of kidney function, and it is in such cases that the Ambard constant should show a variation from the normal if it is to be of any practical value.

Taking the simple formula $\frac{Ur}{\sqrt{\frac{D}{W} \times \sqrt{C}}}$ certain possible defects are

apparent. With a blood-urea within normal limits considerable variations in the factors D and C are necessary in order to produce any marked variation from the normal co-efficient. But if the blood-urea is to be kept down to a normal level, it is evident that the total amount of urea passed must be approximately normal and that the factor D/W will not vary. Secondly, little emphasis is laid on the concentration, for if we take extreme variations of C, as, for instance, 36, 25, 16 and 9 grm. per litre, and keeping the other factors constant at Ur 0·30 and D/W 0·35 work out the corresponding co-efficients, we get as results 0·20, 0·22, 0·25 and 0·30—all well within the normal limit. The formula as above expressed tends to diminish the importance of concentration of urea in the urine, and, by keeping the whole denominator at a fairly constant figure under conditions at all approximating the normal, to lay emphasis merely on the numerator, namely, the concentration of urea in the blood. That such a method could possibly be a delicate indicator of slight variations in kidney functions appears from theoretical considerations alone to be highly improbable, when we consider that it has long been recognised that a low urea concentration is characteristic of certain types of diseased kidney, even when the total amount of urea passed *per diem* approximates to the normal. Nothing is more striking in severe renal lesions than the failure of the kidney to concentrate to an appreciable extent even when the amount of urea in the blood is very high. For instance, in an extreme case the following values were obtained for urea in blood and urine:

Date.	Blood-urea per cent.	Urine-urea per cent.	Number of times urea of blood was concentrated by kidneys.
1:10:'18	0·381	0·997	2·61
5:10:'18	0·461	1·149	2·49
11:10:'18	0·530	1·119	2·11
21:10:'18	0·592	1·208	2·04

Since the normal concentration of urea in the urine is often more than 100 times that of the blood, it is obvious that the function of such a kidney is grossly defective even though a very considerable amount of urea, up to 20 grm. *per diem*, was being excreted.

It is the failure of concentration which is characteristic of the type of renal disease associated with azotæmia, and which, though it may be for a time compensated by an increase in the amount of urine passed, ultimately leads to an accumulation of nitrogenous products in the blood.

The urea concentration test already described furnishes a method by which the inability of the kidney to concentrate urea can be well brought out. Taking six cases of nephritis, in the first three of which retention of blood-urea was present, the following figures were obtained:

		Total urea passed in 2 hours.		Urea concentration in urine.
Group I	(1)	5·56 grm.		1·50 per cent.
	(2)	7·07 „		1·62 „
	(3)	5·94 „		1·26 „
	Average	6·19 „	Average	1·44 „
Group II	(4)	6·34 „		2·42 „
	(5)	5·42 „		2·79 „
	(6)	6·54 „		2·09 „
	Average	6·10 „	Average	2·42 „

It is obvious that while there is no appreciable difference in the *amount* of urea passed by the two groups under the conditions of the experiment, the first three cases in which urea retention was present show a very much lower urea *concentration* than the last three, in which no urea retention had at any time been noted. The more seriously damaged kidneys of Group I excreted the same quantity of urea as the less affected kidneys of Group II, but only by increasing the amount of water passed to an extent adequate to compensate for their defective power of concentration. While following a series of cases of war nephritis we were able to compare the results obtained by the urea test with the figures given by the formula $\sqrt{\frac{D}{W} \times \sqrt{C}}^{Ur}$ and to arrive at some conclusions as to the comparative value of the two tests as indices of the functional activity of the kidneys. The total quantity of urea passed *per diem*, the factor D, was calculated from the amount of urea passed during one hour, and in the course of this hour the patient was bled for the estimation of the blood-urea. This is the method usually followed by many of the advocates of this test, and for many reasons appears to be fairer than actually estimating the total amount of urea excreted during twenty-four hours. The diastatic index and the blood-pressure changes are given as confirmatory evidence of the course of the case. Where no value is given for the urea coefficient the patient was not in a condition to be weighed.

Case 1 :

Date.	Blood-urea per cent.	Urea coefficient.	Diastatic index.	Urea concentration test per cent.
25 : 9 : '15	0·2094	—	5	—
1 : 10 : '18	0·0710	—	6·6	—
8 : 10 : '18	0·0370	0·34	3·3	—
21 : 10 : '18	0·0475	0·46	5	—
8 : 11 : '18	0·0410	0·37	1·4	1·55
6 : 12 : '18	0·0292	0·33	2·2	1·55
27 : 1 : '19	0·0310	0·25	10	2·02

This was a severe case complicated by malaria, but improving rapidly. On discharge there were no cardio-vascular changes. Blood-pressure 120/73. Urine free from protein and containing only a few hyaline casts. The urea coefficient followed the fluctuations in the blood-urea content, and returned to normal limits before a normal concentration was reached with the urea test and while the diastatic index was still low. On discharge the urea concentration had just reached a normal figure, and the diastatic index was well within normal limits.

Case 2:

Date.	Blood-urea per cent.	Urea coefficient.	Diastatic index.	Urea concentration test per cent.
24: 9:'18	0·0715	0·68	1	—
2:10:'18	0·1095	0·85	1	—
17:10:'18	0·1015	0·88	1	—
4:11:'18	0·0945	0·78	1	—
19:11:'18	0·0582	0·46	1	1·27
7:12:'18	0·0474	0·41	1	1·30
23: 1:'19	0·0360	0·36	2	1·37
29: 2:'19	—	—	1·5	1·30

Macroscopic blood was present in the urine until November 26th, 1918. On discharge the blood-pressure was 147/78, the radial artery was definitely thickened, the apex-beat was one quarter of an inch external to the nipple line, while the urine contained a small amount of protein with some granular and hyaline casts. The gradual fall in the blood-urea was associated with polyuria, the patient passing up to 100 oz. per day. The urea coefficient again closely followed the blood-urea, and though the last coefficient estimated was within normal limits, the raised blood-pressure, the cardiac hypertrophy and the persistent low diastatic index did not appear to indicate a very favourable prognosis. In keeping with this the urea test remained at a low figure.

Case 3:

Date.	Blood-urea per cent.	Urea coefficient.	Diastatic index.	Urea concentration test per cent.
31: 8:'18	0·0560	0·46	3·3	—
2:10:'18	0·0373	0·51	4	—
14:10:'18	0·0330	0·47	4	—
13:11:'18	0·0286	0·35	4	1·43
16:12:'18	0·0346	—	2·5	1·33
16: 1:'19	0·0340	0·40	2·2	1·83

On discharge there was still evidence of slight cardiac hypertrophy, the blood-pressure was 145/85, the radial artery being slightly thickened. The

urine was smoky up to October 29th, 1918, and some epithelial casts were present. There was also a considerable amount of œdema.

Here the urea coefficient was with one exception slightly on the high side, in spite of the fact that the blood-urea was within normal limits. When, however, this normal coefficient was obtained, the urea concentration was very low, and the diastatic index was below normal. The urea coefficient in this case tended to be raised, owing to the comparatively small amount of urea passed and the very low concentration usually shown by this patient during the period of the test.

Case 4 :

Date.	Blood-urea per cent.	Urea coefficient.	Diastatic index.	Urea concentration test per cent.
13 : 9 : '18	0·0210	0·38	3·3	—
3 : 10 : '18	0·0678	0·94	2·2	—
25 : 10 : '18	0·0965	0·84	1	—
14 : 11 : '18	0·0745	0·71	1	—
4 : 12 : '18	0·0626	0·54	1	1·13
20 : 12 : '18	0·0468	0·45	1	1·12
24 : 1 : '19	0·0370	0·37	1	1·19

On discharge the apex-beat was external to the nipple line. The radial artery was definitely thickened and the blood-pressure 149 mm. of mercury. The urine gave a faint trace of protein and contained some granular casts. A definite polyuria was present. Phenolsulphonephthalein 18. The urea coefficient followed the blood-urea closely, and the improvement indicated by its fall in the later stages of the illness was not borne out by the diastatic index, the urea concentration test or the clinical findings.

Case 5 :

Date.	Blood-urea per cent.	Urea coefficient.	Diastatic index.	Urea concentration test per cent.
25 : 9 : '18	0·2643	—	6·6	—
1 : 10 : '18	0·1510	—	6·6	—
8 : 10 : '18	0·1180	—	10	—
14 : 10 : '18	0·0625	—	6·6	—
21 : 10 : '18	0·0570	0·43	6·6	—
4 : 11 : '18	0·0700	0·95	6·6	—
19 : 11 : '18	0·0488	0·43	10	1·46
10 : 12 : '18	0·0385	0·44	4	1·37
21 : 1 : '19	0·0325	0·27	1·1	1·41

On discharge the apex-beat was external to the nipple line, the blood-pressure 145/88, and the radial artery was moderately thickened. The urine

contained a considerable amount of protein with a few epithelial and large numbers of hyaline and granular casts. In view of the severity of the attack, the low diastatic index and the clinical findings it did not appear that recovery was as complete as might be inferred from the urea coefficient. This view was in accord with the results of the urea concentration test.

Case 6:

Date.	Blood-urea per cent.	Urea coefficient.	Diastatic reaction.	Urea concentration test per cent.
? :10:'18	0·0334	0·47	2·2	—
28:10:'18	0·0485	0·37	1·6	—
15:11:'18	0·0445	0·48	1·2	1·55
4:12:'18	0·0390	0·41	1	1·09
21: 1 :'19	0·0335	0·37	1·4	1·25

On discharge there were no cardio-vascular changes; the blood-pressure was 122 mm. of mercury. The urine was faintly smoky, and contained a trace of albumen with a few hyaline, granular and epithelial casts. In this case the blood-urea indicated a tendency to retention, and an unfavourable opinion of the kidneys was confirmed by the results of the diastatic index and urea concentration test. The urea coefficient remained at the upper limits of the normal or at a definitely abnormal figure.

These cases represent a fair sample of the results of the various tests as seen in war nephritis and in ordinary civilian nephritis. As will be seen the urea coefficient tends to follow the blood-urea, and gives, in the majority of cases at any rate, no more information than can be obtained by an estimation of the urea in the blood. The results of Keith and Thomson (6), who employed the Ambard coefficient in war nephritis, are very similar, this coefficient usually rising or falling with the urea in the blood. When we compare, however, the normal coefficients obtained in such patients as 2, 4 and 5 on discharge with the other tests of functional efficiency and with the clinical findings, it becomes certain that recovery was not as complete as the urea coefficient would suggest. With a persistently low diastatic reaction and well-marked cardio-vascular changes it is difficult to believe that the kidney had completely recovered its function. In every case the urea concentration test bears out the clinical findings. In our opinion the urea coefficient is of very little, if any, value in cases in which the blood-urea is not raised. When the blood-urea concentration is higher than normal the urea coefficient rises with it, but it is only a waste of time and labour to utilise a complicated mathematical formula when the same information can be obtained from the urea blood concentration alone. In cases in which there is no tendency for the retention of urea the formula gives in the great majority of cases no useful information, and, indeed, tends to

convey the impression that the patient's progress is much more rapid and his kidney efficiency much greater than is actually the case.

For facilities in carrying out this investigation we are indebted to the Medical Research Committee.

CONCLUSIONS.

(1) A new test of renal efficiency is described, depending upon the inability of the diseased kidney to excrete urea at a high concentration.

(2) This test is, in our experience, of more value in indicating deficiency of the kidney function than others now in use.

(3) A criticism of the value of Ambard's "coefficient of urea excretion" is offered.

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TECHNIQUE.

A SIMPLE METHOD OF RECORDING AUTOMATICALLY THE GAS PRODUCED BY BACTERIA IN CULTURE AND OF THE OXYGEN ABSORBED BY AËROBIC NON-GAS-FORMING BACTERIA.

(A Report to the Medical Research Committee.)

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Until the present time there has not been any method described by which the amount of gas produced by the growth of a bacterium can be automatically recorded. Methods have been used by which the gas given off was collected and measured at intervals and so curves of the gas-formation have been made.

The simplest method for collecting and measuring this gas is the one originally described by Beattie (*Brit. M. J.*, 1916, **1**, 756). This consists merely in covering the surface of the implanted culture medium with melted vaseline. The vaseline is allowed to solidify and then the tube is incubated. The gas as it is formed collects under the vaseline plug and pushes it up the tube. The amount of gas can readily be estimated by measuring the distance to which the vaseline plug has been pushed up. This method one of us has used extensively in studying the effect of antiseptics on the growth and gas-formation of microbes in serum. It was felt, however, that it would be a great advantage if the amount of gas could be automatically recorded, thus avoiding the necessity for frequent readings and the possibility of an error in any of the readings. The apparatus described below is the one which we have devised for this purpose. It is simple and can readily be fitted up in any laboratory, as the only piece of apparatus necessary outside of the ordinary laboratory equipment is a slow-moving clockwork drum. The method is best described with reference to Fig. 1 and Fig. 2. Fig. 1 represents the whole apparatus and Fig. 2 represents diagrammatically the arrangement of the culture-tube.

A test-tube (A) containing a culture fluid (B) implanted with gas-forming microbes is taken and a layer of melted paraffin-vaseline mixture (C) is poured on the surface and allowed to solidify. A wooden disc (D) to which a thread is attached is then dropped on the surface and is covered to a depth of 0·5 cm. with the paraffin-vaseline mixture. When this solidifies the wooden disc is firmly embedded in a plug of the paraffin-vaseline mixture about 2 cm. thick. The thread (E) is then carried over a pulley-wheel (F) immediately above the test-tube and fixed to the end of a writing lever (G). This lever, which has its axis on a wheel (H), writes on a smoked clockwork drum (L). At the end of the lever to which the thread is attached there is hung a weight (K).

The whole of the apparatus is placed in the incubator and the gas as it is

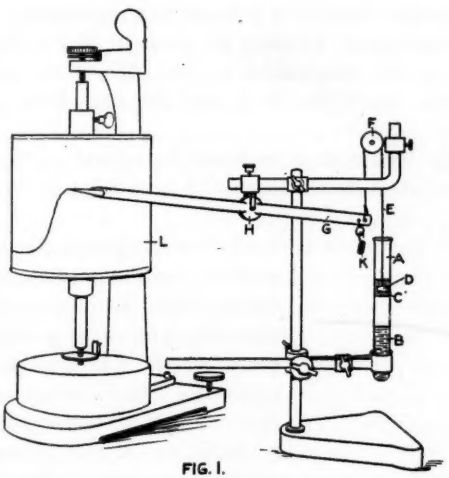
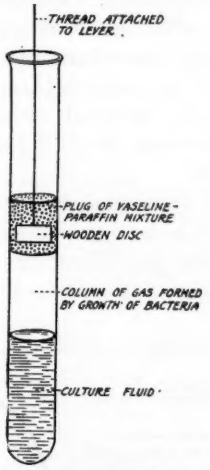


FIG. 1.


THREAD ATTACHED TO LEVER.
PLUG OF VASELINE-PARAFFIN MIXTURE
WOODEN DISC
COLUMN OF GAS FORMED BY GROWTH OF BACTERIA
CULTURE FLUID.

TUBE ARRANGED TO RECORD GAS FORMATION.

FIG. 2.

produced collects under the paraffin plug and pushes it up the tube. As the plug rises the weight attached to the lever will lower that end and so will raise the writing point which is recording on the moving drum. A continuous record of the gas-formation will thus be obtained.

There are certain details to which attention must be paid :

(1) The composition and preparation of the paraffin plug containing the wooden disc. Experiments were made using vaseline, but at incubator temperature this is very soft and the wooden disc is apt to be pulled right out of the vaseline.

Hard paraffin was found unsuitable as it did not slip sufficiently easily inside the test-tube.

The best mixture was found to be 4 parts of vaseline to 1 part of hard paraffin, and this mixture at incubator temperature slipped readily up and

down the tube on the slightest force being applied, and at the same time it was firm enough that the wooden disc would not tear out, even if a comparatively heavy weight was applied.

Before pouring in the paraffin-vaseline mixture the walls of the tube above the culture fluid should be thoroughly dried by heating in the flame, otherwise the plug may not be air-tight.

(2) The weight (K) should be almost heavy enough to counteract the resistance of the paraffin plug to the pressure of the new-formed gas, so that the gas will be approximately at atmospheric pressure. We used weights of about 20 grm.

(3) The writing lever should be strong. There is no advantage in having a delicate lever. We found that corset steels or bicycle spokes made excellent levers when fitted with suitable writing points. The lever should be long, so that the distortion due to its circular motion is reduced to a minimum. Any desired magnification can be obtained by altering the position of the fulcrum (H). We generally used a two-fold magnification, but the most suitable arrangement would depend on the size of the drum and the size of the record desired.

(4) Any type of slow-moving clockwork drum would be suitable. The rate at which the drum should move varies with the type of investigation and the organism experimented with.

Thus, if it is desired to study the latent period before gas-production occurs a drum revolving in twelve or twenty-four hours would be suitable, whereas if a complete record of the gas-formation by a microbe like *B. sporogenes* is desired, a drum revolving once a week or slower would be more convenient. We found that a satisfactory arrangement was to have a 12-hour drum, and from the pin of this we could, by means of a waxed thread, turn another free-running drum. By varying the gearing (which is easily done by rubber bungs of varying sizes attached to the pole of the drum) the second drum could be made to revolve at the rate of from once a day to once a week.

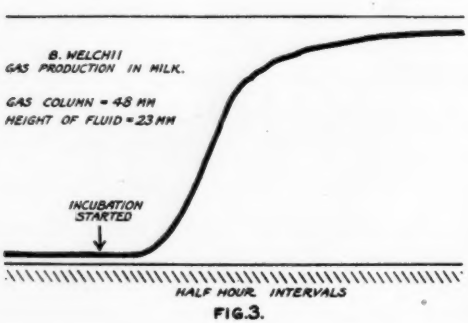


FIG. 3.

It is desirable to have a time marker recording half hours or hours, but if this is not available a mark can be made on the drum a certain number of hours after the commencement of the experiment and the other times calculated from this.

This communication is a purely technical one, so it is unnecessary to go

into the results beyond giving the accompanying typical chart obtained by the method described.

The slight irregularities in the curve are due to slight variations in the temperature of our incubating room, as when there is a column of gas half-an-inch or more high the apparatus acts as an air thermometer. With a perfectly even temperature these irregularities would disappear.

The apparatus can easily be arranged to record the gas-formation in two tubes simultaneously by fixing up two parallel levers connected over two pulley-wheels, with two culture-tubes clamped side by side. By means of this double apparatus observations can be made on the amount of gas formed by two organisms in the same medium, or by the same organism in two different media, or many other comparisons can be made.

When the experiment is concluded the amount of CO_2 in the new-formed gas can easily be measured by pouring on to the surface of the paraffin plug an excess of 10 per cent. caustic soda solution and puncturing the plug so that this comes in contact with the gas. Some of the CO_2 is absorbed and by the caustic soda, and as it is absorbed a negative pressure is created which draws in more soda solution until the whole of the CO_2 is absorbed. The height of the column of gas is measured before and after and the reduction represents the amount of CO.

A similar method can be used automatically to record the oxygen absorption from the air by an aërobic bacterium (Fig. 4). A slight change must here be made as it is necessary to have the air-tight paraffin plug near the top of the tube at the beginning of the experiment. A support for the melted paraffin mixture is obtained by moulding some modelling wax into a thin sheet, and from this (with the top of a test-tube) a disc is cut which is carefully arranged to lie across the tube about 1 in. from the top. The paraffin mixture is poured on the top of this wax disc and then the wooden disc is embedded in it. The arrangement of the rest of the apparatus is as before.

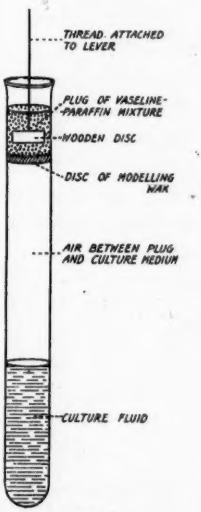


FIG. 4

Tube arranged to record oxygen absorption.

As growth occurs then some of the oxygen will be absorbed from the air of the tube and the plug will be sucked down the tube. This will cause the writing point of the lever to drop.

In conclusion we wish to thank Dr. Roaf for kindly supplying us with the recording apparatus necessary.

A LITMUS SOLUTION SUITABLE FOR BACTERIOLOGICAL PURPOSES.

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PERHAPS litmus is more frequently used than any other substance as a test for the production of acid in media, chiefly in connection with the fermentation of sugars. For this purpose a reliable litmus solution of constant strength is of great value, and an essential if comparable results are desired. Prior to the war it was usual to buy the commercial solutions of litmus. When these were no longer to be obtained it was necessary to prepare them for oneself. None of the various formulæ tried, however, were found very satisfactory. The method given below has, in our hands, given every satisfaction after an extensive and prolonged trial. An important feature of the solution is that it keeps well.

PREPARATION OF THE LITMUS SOLUTION.

Weigh out 80 grm. of litmus granules, and measure out 300 c.c. of 40 per cent. industrial spirit.

To the 80 grm. of litmus in a mortar add 150 c.c. of the spirit. Grind well and then transfer to a flask and boil gently for one minute over gauze. Decant the fluid and add the remainder of the spirit to the granules; boil again for one minute. Decant and add to the first extract. Allow to settle overnight, or centrifuge. Do not filter. Decant into a large measure and make up to 300 c.c. with spirit. Add N/1 HCl drop by drop, shaking from time to time until the colour of the solution becomes purple (usually from 1 to 2 c.c. of the acid to each 100 c.c. of the extract).

The test for the correct reaction is to take a tube of tap-water and a tube of boiled distilled water and add a drop of the solution to each. The tap-water should show blue and the distilled water mauve. When making up media this extract is usually used $2\frac{1}{2}$ per cent.